THE TECTUM MESENCEPHALI OF THE GOLDFISH (CARASSIUS AURATUS)

A light- and electron microscopic investigation

J. Meek

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PROMOTORES : PROF. DR. R. NIEUWENHUYS
PROF. DR. J. J. EGGERMONT
CO-REFERENT : DR. G. VRENSEN

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VOORWOORD

Het onderzoek waarvan de resultaten in dit proefschrift worden beschreven is opgezet en gedurende de eerste jaren uitgevoerd op het Laboratorium voor Medische Fysica van de Universiteit van Amsterdam. Daarna is het onderzoek voortgezet op de afdeling Morfologie van het Interuniversitair Oogheelkundig Instituut te Amsterdam en afgerond op het Laboratorium voor Anatomie en Embryologie van de Katholieke Universiteit te Nijmegen.
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Original objective of the present investigation

The visual system of teleosts is widely used as a model for mammalian and especially human vision research for two main reasons. Firstly, the retina of many teleosts presents a structural and functional pattern basically similar to the human retina, as e.g. appears from the composition by similar layers and cell types, the occurrence of rods as well as cones for dark- and light adapted vision respectively, and the involvement of three types of cones in colour-vision. Secondly, many teleosts are readily available and more easily accessible to experimental manipulation than higher vertebrates (see Schellart, '73).

One of the groups using the teleostean retina as a model in vision research is the "visual system analysis group" at the Laboratory of Medical Physics of the University of Amsterdam, where in particular the retina of the goldfish (*Carassius auratus*) is investigated, in situ as well as isolated. This goldfish-research is combined with fundamental research on human vision, which, in turn, is linked with clinically related system analysis of eye diseases at the Netherlands Ophthalmic Research Institute (N.O.R.I. or I.O.I., i.e. Interuniversitair Oogheelkundig Instituut). After elaborate investigations of the characteristics of retinal ganglion cells in the isolated retina (Spekreyse, '69; Spekreyse et al., '72; Schellart and Spekreyse, '72, '73; Schellart, '73) interest was conceived in the signal processing properties of the target nuclei of these ganglion cells, of which the tectum mesencephali or optic tectum is the most important one. It has been in support of this biophysical investigation of the goldfish tectum that the present histological study has originally been set up and performed.

Literature preceding the present investigation

The tectum mesencephali constitutes the most highly developed part of the brain of fishes (Figs. I.1 and I.2). In most teleosts this structure is differentiated into seven layers which from deep to superficial are indicated as follows: layer 1 or stratum periventriculare (SPV); layer 2 or stratum album centrale (SAC); layer 3 and layer 4, together named stratum griseum centrale (SGC), while layer 4 is also called the inner plexiform layer (IPL); layer 5 or stratum fibrosum et griseum superficiale (SFGS); layer 6 or stratum opticum (SO) and layer 7 or stratum marginale (SM). In goldfish, the thickness of the tectum is about 600 μm. At the time the present investigation started (1974) the following data concerning the structure and function of the teleostean tectum were available.

The retino-tectal projection was analysed by means of degeneration techniques (Sharma, '72a; Vanegas and Ebbesson, '72; Laufer and Vanegas, '74b)

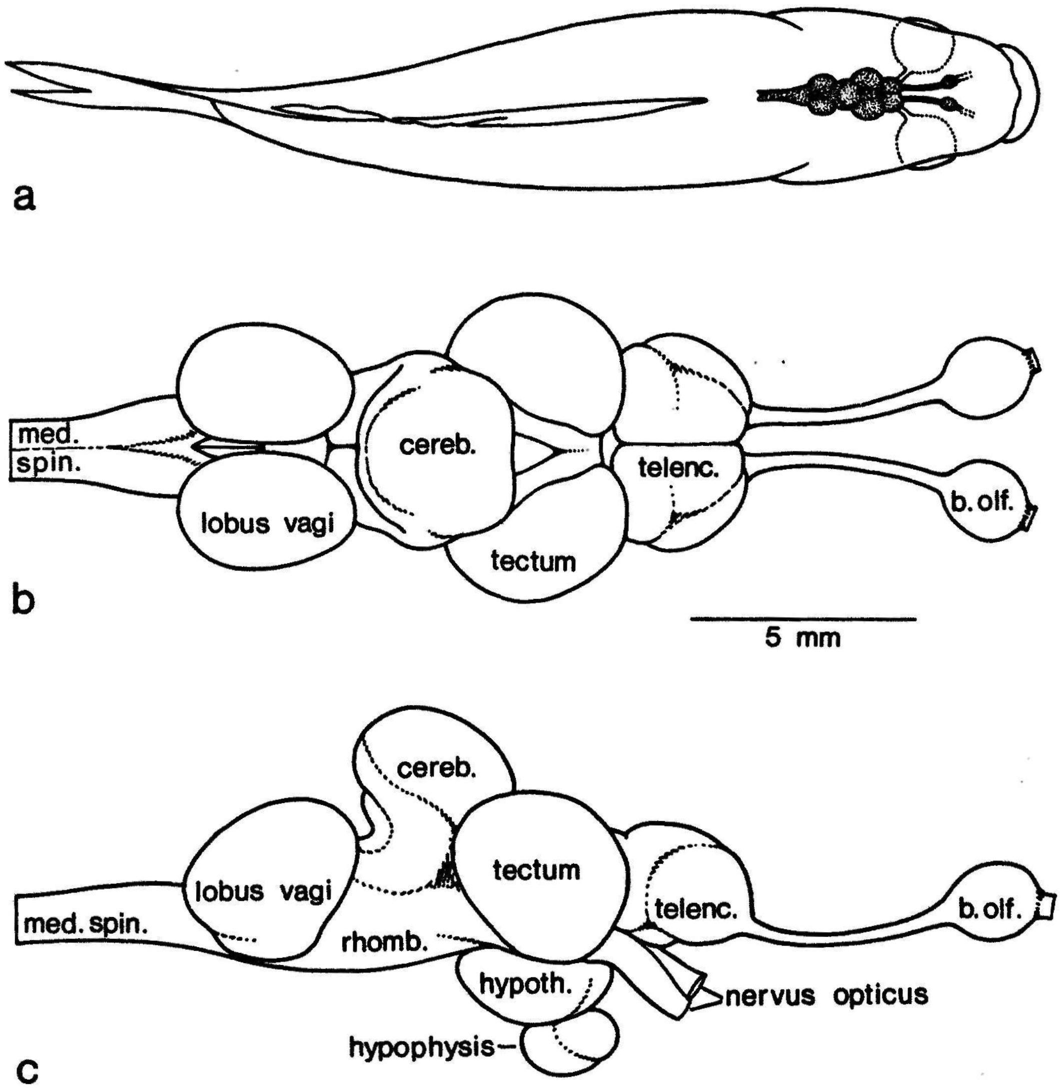

Fig. I.1. The brain of the goldfish. a: *in situ* ; b: dorsal view ; c: lateral view. b.olf.= bulbus olfactorius; cereb.= cerebellum; hypoth.= hypothalamus; med.spin.= medulla spinalis; rhomb.= rhombencephalon; telenc. = telencephalon.

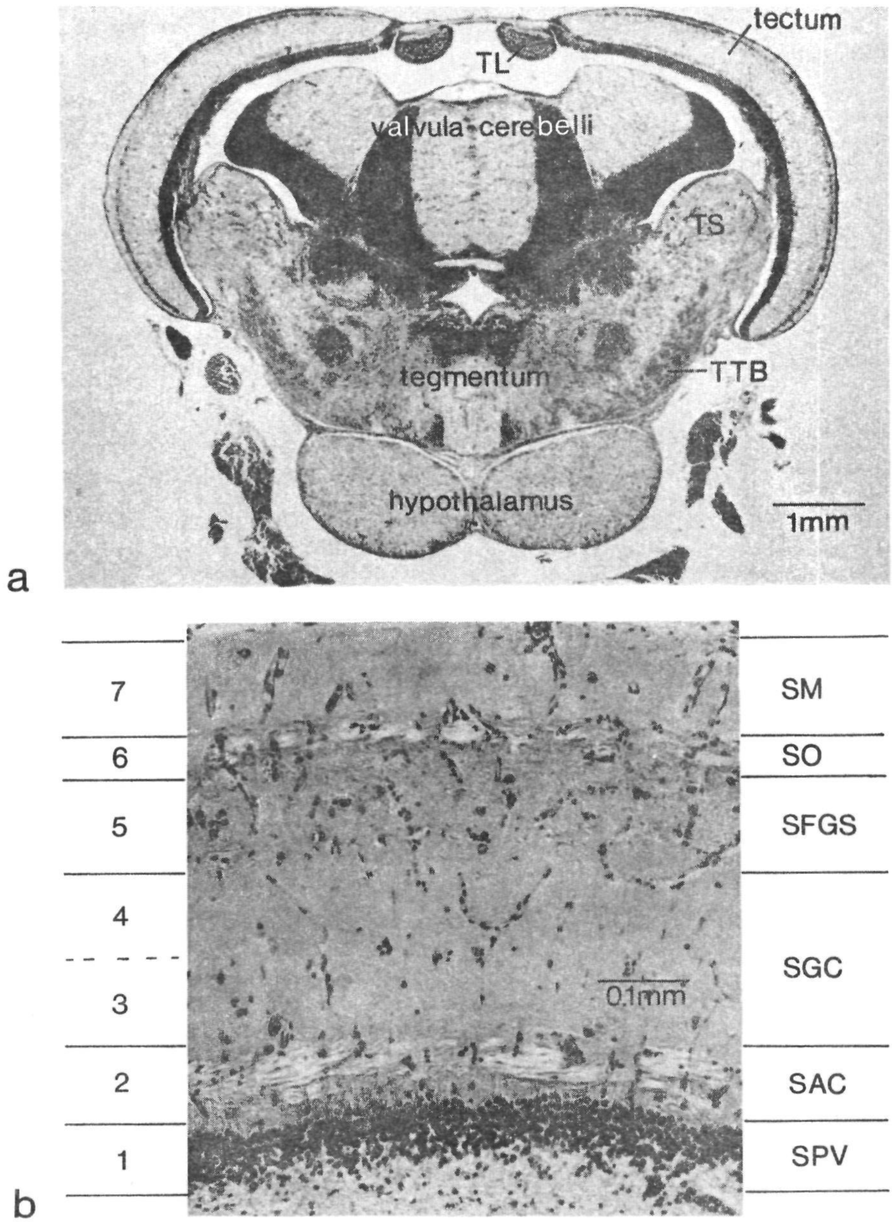

Fig. I.2. Transverse sections through the tectum mesencephali of the goldfish. a: The position of the tectum shown at low magnification (Luxol fast blue-Cresyl fast violet staining).TL= torus longitudinalis; TS= torus semicircularis; TTB= tractus tecto-bulbaris. b: The tectal layers in a section stained with Hematoxilin-Eosin. SAC= stratum album centrale; SFGS= stratum fibrosum et griseum superficiale; SGC= stratum griseum centrale; SM= stratum marginale; SO= stratum opticum; SPV= stratum periventriculare.

and by autoradiographic tracing methods (Grafstein, '67; Neale et al., '72). These studies revealed a massive, exclusively contralateral projection to the SO and SFGS, and a sparse projection, also contralateral, to the SAC. In addition, contralateral as well as bilateral retinal projections to several diencephalic nuclei were described (Ebbesson, '68; Campbell and Ebbesson, '69; Sharma, '72a; Vanegas and Ebbesson, '73; Anders and Hibbard, '74). The existence of precise retinotopic order in the retinotectal projection was known from multi-unit recordings of optic nerve terminals during visual stimulation of the eye with small spots (Buser and Dustardier, '53; Jacobson and Gaze, '64, '65; Schwassmann and Kruger, '65; Kruger, '70) and had let to various regeneration experiments concerning the (chemo-) specificity of this projection (e.g. Jacobson and Gaze, '65; Gaze and Sharma, '70; Yoon, '71; Sharma, '72b). It should be mentioned that the precise retinotopic order found with multi-unit recordings was not confirmed by single unit recordings (Niida and Sato, '72b). The ultrastructural characteristics of the optic nerve were analysed by Hayes ('72) in the goldfish and by Tapp ('73, '74) in the teleost *Eugerres plumieri*.

The first electrophysiological studies of the teleostean tectum concerned tectal evoked responses (TER's), i.e. summated electrical activities of large tectal areas as recorded with low-impedance electrodes. These potentials were studied extensively both after electrical stimulation of the optic nerve (Buser, '49a, b, '50, '51; Buser and Scherrer, '50; Motokawa et al., '58; Konishi, '60b; Vanegas et al., '71a, b, c; Vanegas et al., '74) and after visual stimulation of the eye (Adrian and Buytendijk, '31; Stockfleth Enger, '57; Schadé and Weiler, '59; Konishi, '60a; Schulze, '61; Schadé, '62; Fiedler, '64, '67; Hara et al., '65; Prosser, '65; Peyrethon and Dusan, '67; Ingle, '71). More detailed information was acquired by single unit recording. Most authors used visual stimulation of the eye, with the exception of Vanegas et al. ('74a), who used electrical stimulation of the optic nerve. Many characteristics of the visual tectal units were analysed, including spatial and temporal characteristics (Jacobson and Gaze, '64; Sutterlin and Prosser, '68, '70; Zenkin and Pigarev, '69; Niida and Sato, '72a, b; Guthrie and Banks, '74), spectral selectivity (Jacobson, '64a, b), directional selectivity (Cronly-Dillon, '64; Warzok and Marks, '73),adaptation characteristics (Sato and Niida, '72; Sato, '74) and the sensitivity to the E-vector direction of polarized light (Hashimoto et al., '73; Waterman and Hashimoto, '74; Waterman and Aoki, '74). Visual units appeared to be almost exclusively located in the SO and SFGS, as was determined by electrolytic lesioning of the recording site (Gaze and Jacobson, '63; Jacobson and Gaze, '64; Sutterlin and Prosser, '70; Niida and Sato, '72a; Ramstad and Hughes, '73). Small populations of ipsilateral- (Niida, '73), binocular- (Guthrie and Banks, '74) and multimodal units were described. The latter did not only respond to visual stimulation, but also to lateral line (Callens et al., '67) or auditory stimulation (Niida, '73). In two electrophysiological studies responses of tecto-retinal fibers (Vanegas et al., '73; Sandeman and Rosenthal, '74) were mentioned.

Several histological studies of the teleostean tectum were available in 1974. Early Golgi-studies, of which that of P. Ramon (1899) is the most important one, have been extensively summarized by Ramon y Cajal ('11). Later Golgi studies were published by Leghissa ('55: mainly based on goldfish); Bathelt ('70: *Salmo irideus*) and Vanegas et al. ('74b: *Eugerres plumieri*). These studies revealed a large variety of cell types and a complex cytoarchitecture. Electronmicroscopical studies showed the presence of many types of pre- and postsynaptic elements (Ito, '70; Alley et al., '73; Laufer and Vanegas, '74a) and described the characteristics of neuroglial elements (Kruger and Maxwell, '66, '67). Histochemical studies localized several enzymes and neurochemicals in the various tectal layers (Warzyniak, '62; Kusunoki and Masai, '66). Experimental results concerning tectal connections other than the retino-tectal fibers were not yet available (except for an abstract of Schroeder, '74), but several afferent and efferent tracts were well known from descriptive histological studies (Ariëns Kappers, '21; Ariëns Kappers et al., '36; Schnitzlein, '64; Singh and Khanna, '70).

With respect to general functions ascribed to the tectum of teleosts, visual information processing was by far the most important one. Behavioral studies revealed many visual capacities for teleosts, including the performance of visually guided discrimination-, localization- and orientation tasks (Herter, '29, '30; Hager, '39; McCleary and Bernstein, '54; Saxena, '60; Botsch, '60; Cronly-Dillon and Muntz, '65; Yager, '67; Ingle, '68; Waterman and Forward, '70, '72; Forward et al., '72; Forward and Waterman, '73; Kleerekoper et al., '73; Zuckerman, '73; Zuckerman and Blough, '74). Since the bulk of the retinal fibers projects to the tectum, the role of this structure in the visual information processing necessary for this behavioral capacity was greatly stressed. Apart from the electrophysiological studies described above, evidence for this visual function of the tectum could be derived from comparative anatomical studies relating tectal development with visual specialization (Schmatolla, '72; Winkelmann and Winkelmann, '68), and from the finding that the differentiation of tectal neurons, especially periventricular ones, depends on retinal innervation (Schmatolla, '72). In addition, the RNA and protein synthesis of tectal neurons appeared to be related to visual stimulation (Skrzipek, '69; Kroker, '73).

However, visual information processing was not the only function ascribed to the tectum. The presence of several non optic projections to the tectum (Ariëns Kappers et al., '36; Kirsche and Kirsche, '61; Schnitzlein, '64; Singh and Khanna, '70) as well as the presence of a rather well developed tectum mesencephali in blind fishes (Charlton, '33; Stefanelli, '54; Schmatolla, '62; Alley et al., '73) and deep-sea fish (Shanklin, '34) were considered as an indication for a multisensory integrative function of the tectum. Some electrophysiological results confirmed this idea (Callens et al., '67; Niida, '73). In addition, the tectum was considered as a pre-motor-center, since focal electrical stimulation elicited specific and goal-directed behavioral responses (Chauchard and Chauchard, '27a, b; Akert, '49; Meyer et al., '70; Demski and Gerald, '74). It is remarkable that some authors reported to have found only very slight behavioral changes after tectal ablation (Baudelot, 1864; Dijkgraaf, '49). However, Kirsche and Kirsche ('61) found large disturbances in body posture maintainance and locomotion after removal of one tectal half.

Course of the investigation and framework of this thesis

As has been outlined at the outset, the present morphological investigation of the tectum mesencephali of the goldfish was originally started in combination with a biophysical investigation of the tectum. The purpose of this combined biophysical and histological study was to increase the insight in the functional anatomy of the goldfish tectum, i.e. insight in the relation between morphological and functional characteristics of the constituent neuronal elements. The approach selected to realize this purpose ultimately was intracellular recording with a micropipette and subsequent dye injection, a current technique which enables a combined description of physiological, light- and electronmicroscopical properties of the same neuron (Kater and Nicholson, '73). This type of investigation was a logic continuation of both the retinal research performed at the Laboratory of Medical Physics in Amsterdam and the literature on the teleostean tectum available. However, during this research tectal cells appeared to be not resistant to penetration with a microelectrode, and even extracellular recordings of tectal neurons were difficult to obtain. These technical difficulties have prevented a real coupling between morphology and biophysics. Hence, the results of the biophysical (Schellart and Spekreyse, '76; Riemslag and Schellart, '78; Schellart et al., '79; Ruigt, '79) and histological parts (present thesis) of the project have been published separately.

After a preliminary reconnaissance aimed at the general cytoarchitecture of the tectum and the electrode tip location (see Riemslag and Schellart, '78; Schellart et al., '79) the morphological part of the tectal research was concentrated on an extensive analysis with the Golgi technique. The purpose of this study was to obtain detailed insight in the characteristics of the tectal neurons, which could be used for interpretation of physiological data and to facilitate the identification of intracellularly labelled neurons. This Golgi-study is presented in chapters II and III of this thesis and was published in Stain Technology (Meek, '78) and in the Journal of Comparative Neurology (Meek and Schellart, '78).

Because it appeared to be impossible to stain tectal neurons intracellularly, the most appropriate histological continuation of the Golgi study seemed to be a combined Golgi-electron microscopical analysis of some important tectal cell types with the aid of one of the techniques available at that time (Blackstad, '70, '75; Ramon Moliner and Ferrari, '72, '76; Le Vay, '73; Scott and Guillery, '74; Ito and Kishida, '74; Ito and Atencio, '76; Ribi, '76; Fairén et al., '77). Of these, the gold-toning and deimpregnation technique of Fairén et al. ('77) proved to be the most useful one and was employed for both a qualitative ultrastructural description of six tectal cell types (chapter IVa of this thesis) and a quantitative analysis of their synaptic connections (chapter IVb). These two chapters correspond to publications in the Journal of Comparative Neurology (Meek, '81a, b).

Several aspects, in particular comparative ones, are discussed in chapters III and IV of this thesis. Since a functional interpretation of the present morphological findings was until recently greatly hampered by the absence of experimental results on both tectal connectivity and the electrophysiology of tectal neurons, no attempt has been made to discuss tectal circuitry in chapters III and IV. However, several new relevant data have recently been published (see below). Combination of these data with our own results has yielded some insight in tectal circuitry. This allows for a return to the original purpose of our tectal research in the general discussion (chapter V), where the present knowledge on the functional organization of the teleostean tectum will be discussed and some tentative schemes will be proposed.

Summarizing, the present thesis has the following framework:

1) Chapters II and III deal with the results of a Golgi study of the tectum mesencephali of the goldfish, which has been performed to provide a detailed morphological base for the interpretation of physiological data.
2) In chapter IV the results of the Golgi-study presented in chapters II and III are supplemented with observations at the ultrastructural level, using a combined Golgi-electron microscopical technique. This technique is in particular employed for a quantitative analysis of the synaptic organization of six important tectal cell types.
3) Chapter V is a general discussion, aimed at an exploration of the functional implications of the present and other morphological data.

Recent literature of the teleostean tectum (1975-1981)

The literature about the teleostean tectum available when the present research started (see above), has been supplemented in recent years by a number of important studies. The retino-tectal projection has recently been investigated experimentally in a large variety of teleosts (Gulley et al., '75: *Achirus lineatus*; Landreth et al., '75: *Hemichromis bimaculatus*, *Astronotus ocellatus* and *Carassius auratus*; Repérant and Lemire, '76: *Cyprinus macrolepidotus*, *Cyprinus carpio*, *Alburnus alburnus*, *Tinca tinca*, *Phoxinus phoxinus*, *Rutilis rutilis*, *Scardinus erythrophthalmus*, *Esox lucius*, *Perca fluviatilis*, and *Salmo irideus*; Voneida and Sligar, '76: *Astyanax hubbsi* and *Astyanax mexicanus*; Springer and Landreth, '77: *Carassius auratus*;

Luckenbills-Edds and Sharma, '77: *Pseudopleuronectus americanus*; Peyrichoux et al., '77: *Alburnus alburnus, Tinca tinca, Scardinus erythrophthalmus, Leuciscus rutilus* and *Rutilis rutilis*; Pinganaud and Clairambault, '79; *Salmo irideus*; Schmidt, '79: *Carassius auratus*; and Meyer and Ebbesson, '81; *Synodontis nigriventris*), confirming the well known pattern of an extensive contralateral projection to the SO and SFGS, a sparse contralateral projection to deeper layers, especially SAC, and several additional retinal projections to diencephalic nuclei. Moreover, it has been shown experimentally that the tectum receives afferents from several other sources: the telencephalon (Vanegas and Ebbesson, '76; Ito and Kishida, '77; Grover and Sharma , '81; Luiten, '81), the torus longitudinalis (Ito and Kishida, '78), the contralateral tectum (Ebbesson and Vanegas, '76; Sligar and Voneida, '76; Grover and Sharma, '79, '81; Luiten, '81), the nucleus isthmi (Grover and Sharma, '81; Luiten, '81), which is, like the retino-tectal projection, topographically organized (Ito et al., '81a; Sakamoto et al., '81), several pretectal and thalamic nuclei, the torus semicircularis, and nuclei of the mesencephalic and rhombencephalic reticular formation (Grover and Sharma, '81; Luiten, '81).

Tectal efferents have recently been investigated with degeneration techniques (Ebbesson and Vanegas, '76; Sligar and Voneida, '76; Grover and Sharma, '79) and with anterograde HRP transport (Grover and Sharma, '81; Luiten, '81). These studies revealed the presence of ascending, commissural, and descending efferent tectal tracts. The tectal targets include: several pretectal nuclei, the so-called lateral geniculate nucleus and the nucleus rotundus in the diencephalon; the torus longitudinalis, the torus semicircularis, the contralateral tectal half and the nucleus isthmi in the mesencephalon and the mesencephalic and rhombencephalic reticular formation. In addition, Schmidt ('79), Meyer and Ebbesson ('81), and Meyer et al. ('81) have described tecto-retinal fibers. Several types of efferent tectal neurons have recently been labeled by means of HRP injection in a number of tectal targets or efferent tracts, including the retina (Schmidt, '79) the nucleus isthmi (Ito et al., '81b), the pretectal region, the contralateral tectum, the torus semicircularis and the tectobulbar tract (Grover and Sharma, '81). Taking together the results of all of these recent hodological studies it may be stated that the tectal connectivity appears to be much more complicated than was known at the time our research was started.

Concurrent with our own Golgi-study on the goldfish tectum (chapter II and III) other authors published Golgi-studies on the teleostean tectum as well, using the siluroids *Bagrus sp.* and *Ictalurus punctatus* (Schroeder and Vanegas, '77), the goldfish *Carassius auratus* (Romeski and Sharma, '79), the jewel fish *Hemichromis bimaculatus* (Coss and Globus, '79), the trout *Salmo irideus* (Pinganaud and Clairambault, '79) and the squirrel fishes *Holocentrus rufus* and *H. ascensionis* (Schroeder et al., '80). In addition, Kishida ('79) published a comparative Golgi-study based on a large variety of teleostean species. All of these studies reveal that the tectal cell types occurring in different species are basically similar, but that considerable quantitative differences with respect to dendritic elaboration and extension may occur between different species.

Only few electron microscopical studies on the teleostean tectum were recently published (Ciani et al., '75; Marotte and Mark, '75; Airhart, '79; Airhart and Kriebel, '80; Ito et al., '80). The most extensive paper is based on *Holocentrus rufus* (Ito et al., '80) and deals with the classification and quantification of presynaptic elements. In all teleosts studied so far, retinal afferents appear to be distinguishable from other presynaptic elements by their large round vesicles and pale mitochondria with dilated cristae (Ito, '70 (*Cyprinus carpio*); Laufer and Vanegas, '74b (*Eugerres plumieri*); Airhart and Kriebel, '80 (*Carassius auratus*) and Ito et al., '80 (*Holocentrus rufus*). These features were gratefully used to interpret some of the present Golgi-EM results (chapter IV). Presynaptic terminals of afferents from the telencephalon (Airhart, '79; Ito et al., '80) and the contralateral tectum (Ito et al., '80), however, can not be identified on the basis of morphological criteria.

Recent electrophysiological tectal studies deal predominantly with spatial and temporal characteristics of extracellularly recorded visual units, of which many types have been described (Galand and Liege, '75; O'Benar, '76; Schellart and Spekreyse, '76; Guthrie and Banks, '76, '78; MacKeben, '77; Riemslag and Schellart, '78; Schellart et al., '79). Two groups have succeeded in intracellular recording of visual units in the teleostean tectum and subsequent identification by means of dye injection. Niida et al. ('80) found in the crucian carp (*Carassius carassius*) predominantly pyramidal neurons in the SFGS, which showed a remarkably large variety of responses to visual stimuli. Rowe and Beauchamp ('79) and Rowe ('80) could identify in the rock bass (*Ambloplites rupestris*) a type of neuron in the stratum marginale with peculiar morphological as well as physiological properties (the so-called on-off burst cells). Other recent electrophysiological tectal studies include the field potential analysis of Schmidt ('79), the study of Vanegas et al. ('79) of the toro-tectal marginal fibers and their target cells, and an investigation of the electric properties of some tectal targets, in particular the nucleus isthmi and nucleus prethalamicus (Williams and Vanegas, '81).

Several recent studies deal with tectal histochemistry, predominantly related to the presence of acethylcholine-esterase (AChE) (Contestabile and Zannoni, '75; Contestabile, '76a, b, '78; Villani et al., '79; Marani and Ruigrok, '81). A number of authors have presented evidence for acetylcholine to be the retinotectal neurotransmitter in teleosts, working via nicotinic cholinergic receptors (Francis and Schechter, '79; Schechter et al., '79; Freeman, '79; Oswald et al., '80; Schmidt and Freeman, '80; Schwartz et al., '80). Other studies indicate, in addition, the presence of intrinsic cholinergic circuits involved in the visual information processing in the tectum (Contestabile et al., '78, '79; Migani et al., '80). These intrinsic circuits probably use muscarinic cholinergic receptors (Francis and Schechter, '79; Francis et al., '80). Intrinsic GABA-ergic (Villani et al., '81) and glutamatergic (Francis et al., '81) circuits appear to be present in the tectum of the goldfish as well.

To complete this brief review of recent tectal literature the following studies may be mentioned. The effect of electrical tectal stimulation was studied by Demski and Bauer ('75), whereas Yager et al. ('77) and Springer et al. ('77) made detailed observations on the effect of tectal ablation. The latter authors showed that some types of behavior disappeared (optomotor response; food pellet localization; shadow induced deceleration of respiration), whereas others were not influenced (optokinetic nystagmus and the dorsal light reflex). Coss and Globus ('78, '79) and Burgess and Coss ('80) described the effect of social stimulation on tectal neurons in the jewel fish (*Hemichromis bimaculatus*). Isolation as well as crowding change the number of spines and dendritic branches and spine morphology of the spiny piriform periventricular neurons. The many papers published in relation to the plasticity and specificity of the retino-tectal system in the goldfish will not be mentioned here. An extensive review has been published by Horder and Martin ('78).

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MYELIN IMPREGNATION: AN IMPROVED GOLGI-COX MODIFICATION

J. MEEK, *Laboratory of Medical Physics, University of Amsterdam, Amsterdam, The Netherlands*

ABSTRACT **Optic tecta of goldfish were coated with egg yolk and immersed for only one week in one of the following impregnation fluids: a) Solution A + B; A = 1 g $K_2Cr_2O_7$ and 1 g $HgCl_2$ boiled for 15 min in 85 ml distilled water and allowed to cool; B = 0.8 g $K_2Cr_2O_4$ and 0.5 g KWO_4 dissolved in 20 ml distilled water. b) Solution A + B two volumes diluted with boiled distilled water. c) Solution A + B four volumes diluted with boiled distilled water. Each tectum was immersed 6 hr in 100 ml distilled water containing 0.5 g LiOH and 15 g KNO_3, washed 18 hr in 500 ml 0.2% acetic acid, dehydrated with ethanol, and embedded in low viscosity nitro cellulose. Sections were cut at 100 μm with a rotary microtome after clearing with cedarwood oil. Methods b) and c) have two advantages compared with method a), the original Golgi-Cox method. First, more cells are impregnated, especially in the layers extending 200–400 μm below the surface, and dendrites as well as unmyelinated axons are well impregnated. Second, myelin sheaths are impregnated and can be recognized by their peculiar chain-like appearance. The described Golgi-Cox modification offers an appropriate method to study the morphology of superficially located nervous tissue.**

The Golgi-Cox technique of Ramón-Moliner (1958, 1970) is less than optimal for impregnation of superficially located neurons. Although the modification of Schroeder (1973), who coated tissue with egg yolk before impregnation, improved its applicability for a superficial structure like the fish tectum, I observed that cells in the superficial and intermediate layers of goldfish optic tectum still were seldom or poorly impregnated. The impregnation properties of this Golgi-Cox technique could be improved greatly, however, by limiting the impregnation period to one week and especially by diluting the impregnation fluid as described in this paper.

METHODS

Goldfish 18–25 cm in length were anesthetised with MS222 (0.3 g/l, 10 min) and the tectum was exposed by removing part of the skull and fatty tissue. Next the tectum, together with thalamus and hypothalamus, was removed and dropped in egg yolk for coating (Schroeder 1973). Coated tecta were immersed in 50 ml of one of the following impregnation fluids.

a) Golgi-Cox fluid prepared according to Ramón-Moliner (1958, 1970). First, 1 g potassium dichromate and 1 g mercuric chloride were dissolved in 100 ml distilled water. This solution (A) was boiled for 15 min and, after cooling, distilled water was added until the final volume was 85 ml. Next, 0.8 g potassium chromate and 0.5 g potassium tungstate were dissolved in 20 ml distilled water (solution B). Finally, solution B was added to solution A.

b) One-half Golgi-Cox fluid. First the original Golgi-Cox fluid was prepared according to a). This fluid was added to an equal volume of boiled distilled water to obtain the final impregnation fluid.

c) One-quarter Golgi-Cox fluid. The original Golgi-Cox fluid was now diluted by four volumes in boiled distilled water.

The impregnation period was shortened to one week for all fluids. This reduced the amount of nonspecific precipitate and impregnation artefacts; however, this period still appeared sufficiently long to impregnate cells completely.

After impregnation, the coated tecta were treated according to the rapid procedure of Ramón-Moliner (1970) as follows: 6 hr immersion in 100 ml distilled water containing 0.5 g lithium hydroxide and 15 g potassium nitrate, 18 hr rinsing in 500 ml distilled water containing 1 ml concentrated acetic acid, dehydration with ethanol, and embedding in low viscosity nitrocellulose (LVN). Sections of 100 μm were cut with a rotary microtome according to the dry method of Walls (1936). For this purpose hardened LVN blocks with tissue were soaked for 24 hr in a mixture of cedarwood oil and chloroform (1:1) and then for three days in pure cedarwood oil.

Results and Discussion

The first advantage of methods b) (Fig. 1) and c) is that impregnated cells frequently are visible in the stratum griseum centrale and stratum album centrale, extending 200–400 μm below the surface, whereas with method a) no impregnated cells can be found in these layers. The dendrites of these cells are well impregnated and can often be followed for long distances (up to 1000 μm), while unmyelinated axons also can be traced in these layers (Fig. 2; Meek and Schellart 1978). Unfortunately, the most superficial 200 μm remained poorly impregnated (stratum marginale, stratum opticum and the superficial part of the stratum fibrosum et griseum superficiale).

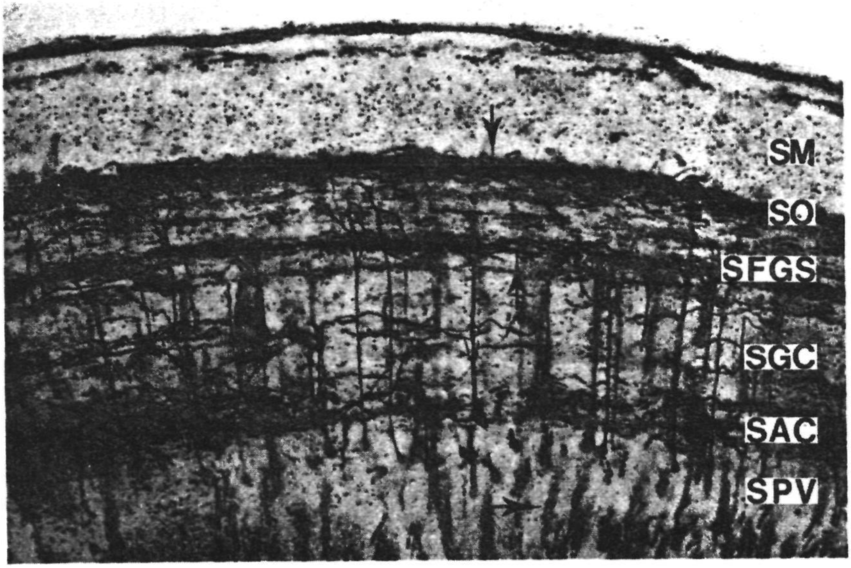

FIG. 1. Goldfish optic tectum impregnated with ½ Golgi-Cox fluid. Arrows point to layers with myelinated axons. SM = Stratum marginale. SO = Stratum opticum. SFGS = Stratum fibrosum et griseum superficiale. SGC = Stratum griseum centrale. SAC = Stratum album centrale. SPV = Stratum periventriculare. × 105.

FIG. 2. Periventricular neuron with an unmyelinated axon (arrows) impregnated with ½ Golgi-Cox fluid: s = soma, ds = dendritic shaft, d = dendrite. × 345.

The second and most important advantage of methods b) and c) is that several axonal courses become visible by impregnation of a peculiar chain-like structure, which is more delicate in ¼ Golgi-Cox material than in ½ Golgi-Cox material (Figs. 4 and 5 respectively). Comparison with luxol fast blue myelin staining (Klüver and Barrera 1953) revealed that these structures are impregnated myelin sheaths since their general location and course (Fig. 1, arrows) agrees with the myelin distribution in material stained according to Klüver and Barrera (1953) and since the characteristic courses of the myelinated axons of some cells, as shown in Figs. 3, 4 and 5, are also found with Klüver and Barrera staining (Figs. 6 and 7 respectively). Especially the arciform myelin sheaths (Fig. 6) provide a strong indication that the chain-like structures in our Golgi material are myelin sheaths since no other structures with such a course are found in the goldfish tectum (Meek and Schellart 1978).

In conclusion, the described Golgi-Cox modification offers an improved method to study superficially located parts of the brain and enables investigation of both the dendrites as well as the axons of neurons. The latter holds particularly well for neurons with a myelinated axon.

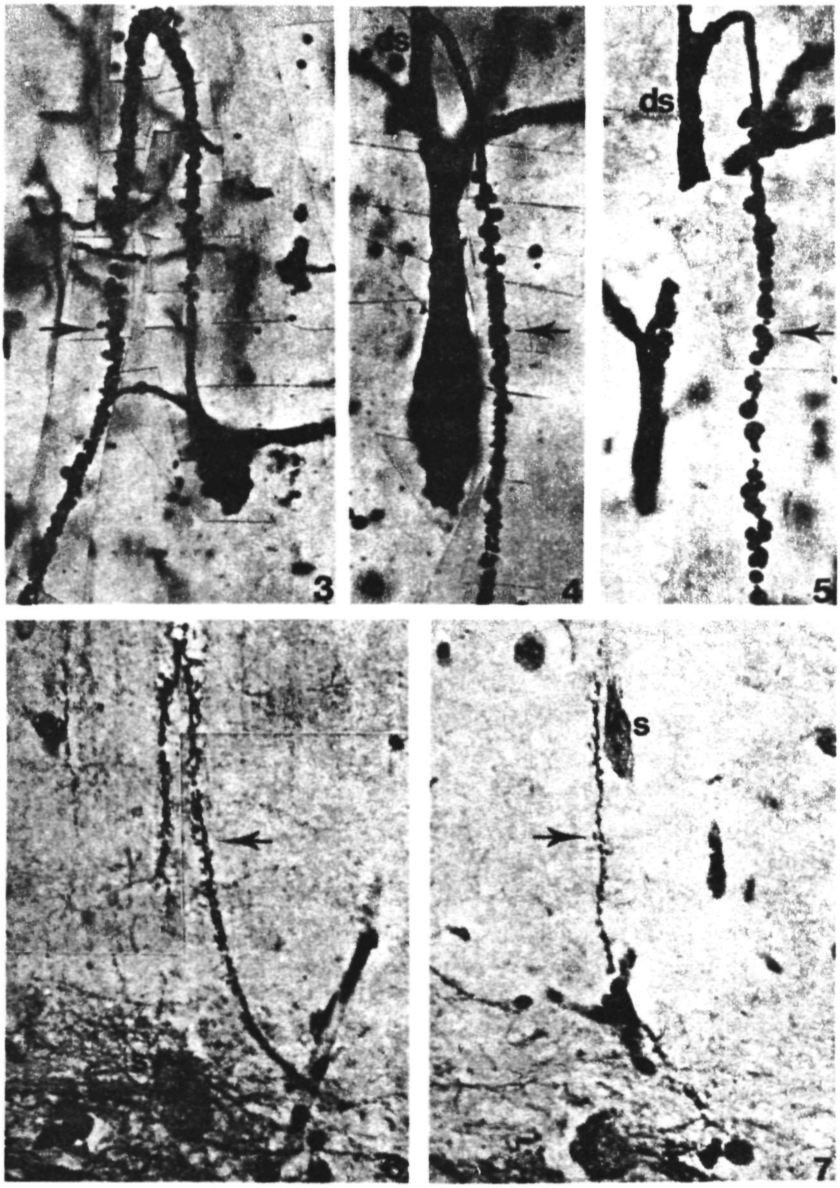

FIG. 3. Detail of a neuron with an arciform myelinated axon (arrow) impregnated with ½ Golgi-Cox fluid. × 410.

FIGS. 4 and 5. Details of neurons with a recurrent myelinated axon (arrows) arising from the apical dendritic shaft (ds). Fig. 4: ¼ Golgi-Cox fluid, × 840. Fig. 5: ½ Golgi-Cox fluid, × 940.

FIGS. 6 and 7. Details of the same type of neurons as shown in Figs. 3 and 4, respectively, stained with luxol fast blue and cresyl fast violet. Arrows point to myelin (blue stained). s = soma (violet stained). × 460.

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A Golgi Study of Goldfish Optic Tectum

J MEEK AND N A M SCHELLART
Laboratory of Medical Physics University of Amsterdam 1016 BS Amsterdam The Netherlands

ABSTRACT A study of goldfish optic tectum was performed with rapid Golgi, Golgi-Kopsch and a modified Golgi Cox impregnation which proved quite suitable to impregnate cells in the middle tectal layers and to study more close ly axonal properties Fifteen cell types are distinguished, based upon the position of dendritic trees and axonal properties Two cell types are found with den drites in the marginal layer type I with an axon terminating in the central gray layer and type II without an impregnated axon Three cell types (III, IV and V) have dendrites in a single, specific tectal layer and an axon terminating within the tectum Five cell types (VI X) have dendrites in two horizontal planes Two of them have myelinated axons leaving the tectum, whereas the axons of the remaining three types project to different tectal layers While these first ten cell types have dendrites almost exclusively in the superficial half of the tectum, the remaining five types have dendrites in deeper layers too This especially holds for the most conspicuous tectal cells (types XII and XIII), which have dendritic trees branching at three or more horizontal levels and a myelinated axon leaving the tectum, with sometimes a very peculiar course (XIII$_1$) Also type XI has three or more dendritic trees, but its axon was not found The numerous cells with cell bodies in the deepest tectal layer (type XIV) have dendrites and axonal terminations anywhere in the tectum, except in the most superficial and the deepest layer However, most dendrites occur in the optic layers, whereas the axons, always originating from the dendritic shaft in the superficial tectal half, generally terminate in the middle tectal layers Type XV cells have their soma in the deepest tectal layer as well, but their den drites do not reach the optic layers Per tectal lobe the following numbers are estimated type I 5,000-20,000 neurons, Type III 2,500 10,000, types IV - XIII each 500-2,000 and type XIV 1,000,000-2,000,000 The total number of myelinated tectal efferents is estimated at 2,000-8,000 Comparison with other Golgi studies in teleosts leads to the conclusion that the tecta of these species of fish are basically similar

In the brain of fish, the optic tectum plays an important role in visual information processing The electrophysiology of this brain center has been investigated in several species, like the lagoon fish *Eugerres plumieri* (Vanegas et al , '71, '74a), the pike *Esox lucius* (Zenkin and Pigarev, '69) and the crucian carp *Carassius carassius* (Niida and Sato, '72) Most studies deal, however, with goldfish, *Carassius auratus* (e g , Jacobson and Gaze, '64, Sutterlin and Prosser, '70, Watermann and Aoki, '74, Schellart and Spekreijse, '76)

Following the early Golgi studies of fish tectum, summarized by Ramon y Cajal ('11), the histology of goldfish tectum was examined extensively by Leghissa ('55) Leghissa distinguished 13 cell types based upon shape, polarity, size and location of the soma These cell types can also be distinguished in respect to axonal properties The axons of six cell types leave the tectum via the deep fibrous tectal layers, two other types send their axons to the superficial fibrous layer, and the axons of the remaining cell types terminate either within the tectum or are not described Since Leghissa used other fish species too, his study is not so much an exact description of goldfish tectal cell types, but presents the general cytoarchitecture of fish tectum After the work of Bathelt ('70) on trout tectum *(Salmo irideus)*, and of Ito ('70) on carp tectum *(Cyprinus carpio)*, Vanegas et al ('74b) published the most detailed Golgi study on fish optic tectum In this study on *Eugerres plumieri* (see also Vanegas, '75) about 11 cell types were distinguished on the basis of somatic and dendritic properties In gross features this description resembles that of Leghissa ('55), but at cellular level there are substantial discrepancies A recent study of Schroeder and Vanegas ('77) on two siluroid teleosts revealed basically the same cell types as found in *E plumieri*

Autoradiographic techniques (Grafstein, '67 Neale et al, '72, Landreth et al, '75) as well as optic nerve degeneration studies (Sharma, '72) revealed that the optic nerve fibers of goldfish terminate in the plexiform and gray layer underneath the optic fiber layer The same was found for *E plumieri* by Vanegas and Ebbesson ('73) In the rostral part of the tectum, optic nerve fibers can also be found in the deepest fibrous layer (Sharma, '72)

The present paper intends to provide a histological base for physiological studies on goldfish tectum Leghissa's study ('55) is not so appropriate for this purpose since it gives an exemplary description of cellular characteristics and since some of his findings are not in line with the data of Vanegas et al ('74b) Special attention will be paid to the layers of dendritic termination, the horizontal dendritic extension, the level of origin of the axons, the axonal course and projection, the position of the cell bodies and the number of cells per cell type These data will also provide a base for further electron microscopic investigation, which is feasible in combination with Golgi-impregnations (Blackstad, '75, Ramon-Moliner and Ferrari, '76, Fairen et al, '77)

METHODS

Common goldfish *(Carassius auratus)*, 18-25 cm long, were used After exposure of the tectum of anaesthetized fish (MS 222, one-third g/l, 10 minutes) the animal was sacrificed by cutting the medulla Within the next minute the tectum was removed, together with other mesencephalic structures and immersed in a fixative After impregnation by a Golgi method the tectum was embedded in 30% low viscosity nitrocellulose (LVN) according to the method of Clark and Clark ('71), sectioned with a rotary microtome according to the rapid celloidin method of Walls ('36) and mounted in Depex (Gurr 85900) Section thickness was 30 μm for rapid Golgi, 75 μm for Golgi Kopsch and 100 μm for Golgi Cox material

The following Golgi impregnation methods were applied

(1) *Rapid Golgi (GR)* 44 hours fixation in 2% potassium dichromate plus 0 2% osmium tetroxide followed by 20 hours impregnation in 0 75% silver nitrate (modified after Humason, '72)

(2) *Golgi Kopsch (GK)* Four or five days fixation in 1 5% potassium dichromate plus 5% glutaraldehyde followed by one or five days impregnation in 0 75% silver nitrate (modified after Stone and Freeman, '71)

(3) *Golgi-Cox (GC)* Several modifications were applied based on the methods of Ramon Moliner ('58, '70) According to this method solution A contained 1 g potassium dichromate and 1 g mercuric chloride, dissolved in 85 ml distilled water and boiled for 15 minutes, and solution B, 0 8 g potassium chromate and 0 5 g potassium tungstate dissolved in 20 ml distilled water The tecta were coated with egg-yolk (ey) according to Schroeder ('73) and after impregnation the rapid procedure of Ramon-Moliner ('58, '70) was applied up to the fourth stage The four modifications used are

a Long GC + ey Two or four weeks impregnation in solution A + B

b GC + ey One week impregnation in solution A + B

c ½ GC + ey One week impregnation in a fluid consisting *half* of solution A + B and *half* of boiled distilled water

d ¼ GC + ey One week impregnation in solution A + B diluted in three times the volume of boiled distilled water (final concentration ¼)

Impregnation times longer than one week did not improve the impregnation The quality sometimes even decreased, except for some superficial cell types, which could only be impregnated by means of procedure 3a Generally 3c and 3d gave the best results of the GC modifications (Meek, '78)

Sections stained with Hematoxilin-Eosin (Humason, '72) or with Luxol fast blue-Cresyl fast violet (Kluver and Barrera, '53) were used in order to estimate cell densities

To determine the position of the cells in the tectal layers basically the classifications of Leghissa ('55) and Vanegas et al ('74b) were used. However, especially in the location of layer 4 (Inner plexiform layer, IPL), these two studies seem to differ due to the fact that Vanegas et al. ('74b) describe a border where Leghissa ('55) defines the center of this layer (fig. 1).

We used the same borders as Vanegas et al. ('74b) with their IPL indicated as a distinct layer (layer 4), resulting in seven layers, numbered from inside outwards according to Leghissa ('55) (center column of fig. 1). Structures just in the border region between two layers X and Y will be indicated as being positioned in layer X/Y (fig. 1). Thus, the fibrous layers that Leghissa ('55) indicates with A, B, C, D and E can be compared with our layers 2, 3/4, 4/5, 5/6, and 6 respectively.

A Leitz Ortholux II microscope with a Leitz drawing apparatus was used to study the sections. If necessary, adjacent sections (up to 6) were used to obtain complete drawings of the cells. In this way about 575 neurons of 11 impregnated tecta were drawn, while 14 other tecta were used for comparison. Horizontal, sagittal as well as transverse sections were studied. It was attempted to distinguish in the drawings different cell types, primarily based upon the location of the dendritic trees in the tectal layers. Since these locations can vary substantially and dendritic trees are occasionally not impregnated, we checked quantitatively whether the dendritic locations were consistent for a given cell type. Hereto in each drawing the tectum was divided in 29 zones parallel to the surface and equal in thickness so that layers 1, 2, 3, 4 and 7 each contained four zones, layer 6 three and layer 5 six zones. Next, for each cell the horizontal extension of dendritic branches was measured per zone and for each cell type the mean values were plotted as a function of the vertical position. In figure 2 this procedure is illustrated by two cell types with a large variation in their dendritic location. From such figures it can be concluded how many dendritic trees are typical for a certain type and where they are located. Furthermore, the location of any particular tree can vary. For instance, the basal dendritic tree of the second cell type of figure 2 can have a position ranging from the

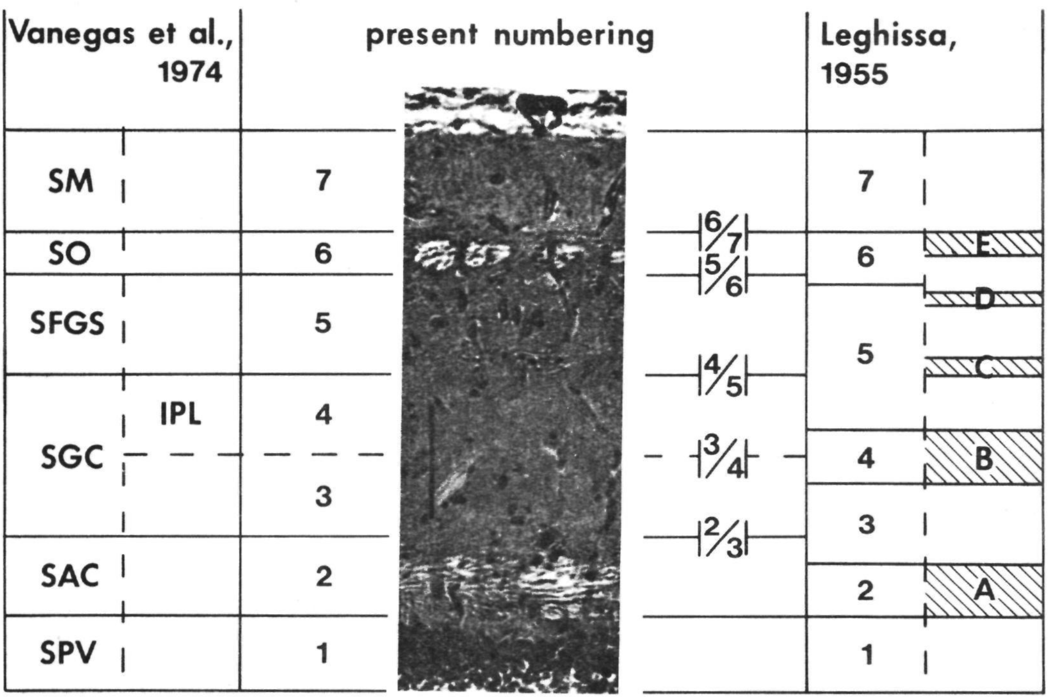

Fig. 1 The tectal layers, as indicated by the authors mentioned. The photograph shows a tectum stained with Hemataxilin-Eosin. SM, Stratum marginale; SO, Stratum opticum; SFGS, Stratum fibrosum et griseum superficiale; SGC, Stratum griseum centrale; SAC, Stratum album centrale; SPV, Stratum periventriculare; IPL, Inner plexiform layer; A-E, plexiform layers. Calibration bar, 100 μm.

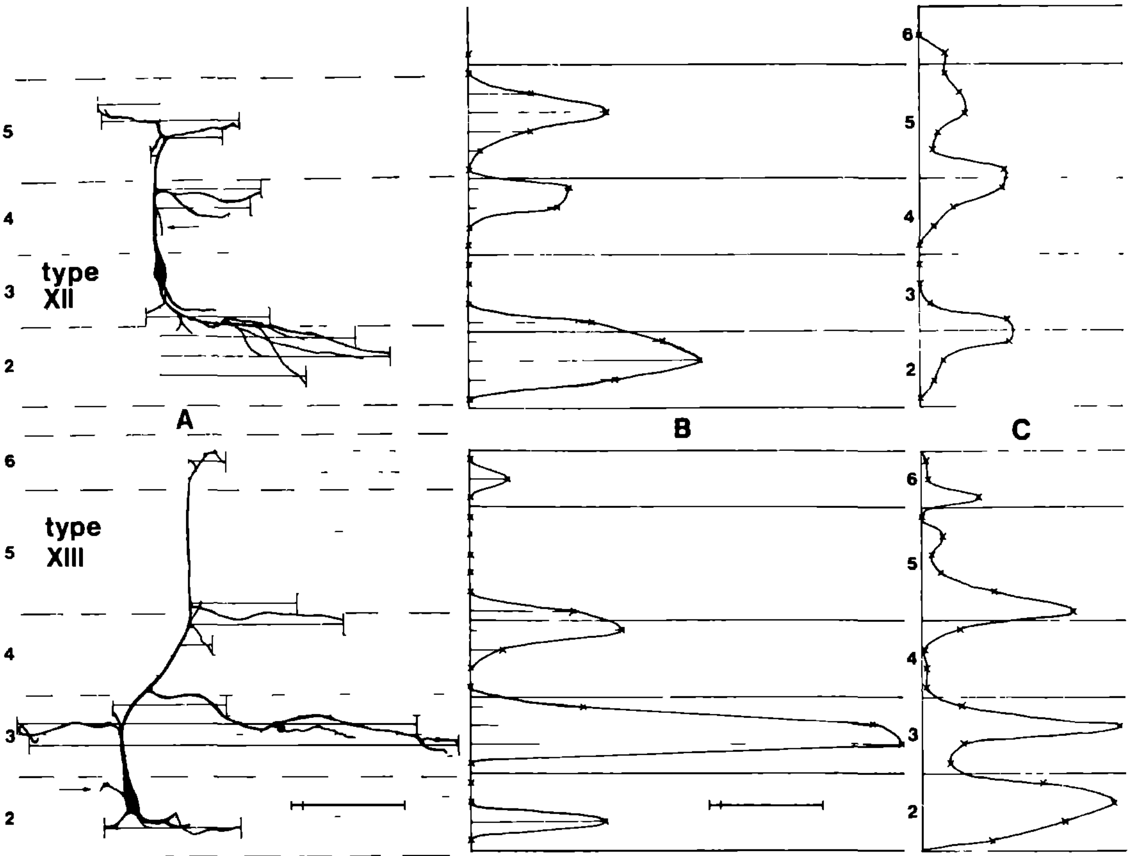

Fig. 2 The method used to quantify the dendritic tree position, illustrated for two cell types (XII and XIII).

A In the drawings of individual neurons the tectum is divided in 29 zones (see text) and in each zone the horizontal extension of the dendrites is measured.

B The dendritic extension of the cell drawn is plotted against the vertical position.

C The average plot of all 30 type XII and all 32 type XIII cells studied.

Arrow indicates axon, calibration bar, 100 μm (first division, 10 μm).

upper to the lower part of layer 2. However, all these trees are regarded as homologous, since their distribution gives rise to a single peak. By taking all homologous individual trees together the mean horizontal extension and standard deviation were estimated (table 3). It should be noted that these values are obtained from drawings, and consequently present the extension projected on the plane of sectioning. The standard deviations of these values reflect partly biological variation, partly methodological imperfection. Imperfections like incomplete impregnation, abrupt ending of dendrites at the edge of a section without recovery in adjacent sections and the fact that the dendrites measured may run at a certain, unknown angle with respect to the plane of sectioning, cause an underestimation of the mean value of the horizontal extension. The maximal observed value given in table 3 will be less influenced by these imperfections.

RESULTS

Morphological characteristics of tectal cells

In this study tectal cells are divided into 15 types, numbered from I to XV in such a way that roughly the higher the number, the deeper the soma is located in the tectum. Furthermore, type III-V cells are monostratified (with dendrites at a single level), type VI-X cells are bistratified (with dendrites at two separate levels) and type XI-XIII cells are multistratified. Types XIV and XV contain all

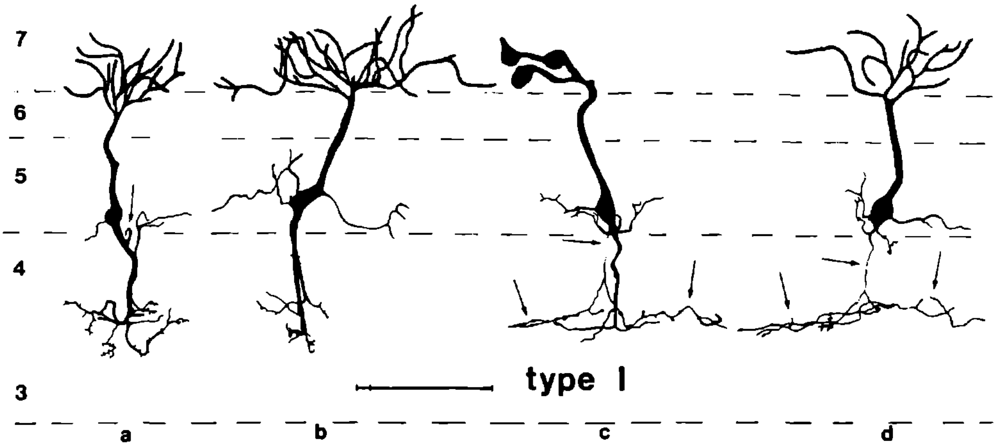

Fig. 3 Type I cells. In this and subsequent figures the arrows point to the axons and the calibration bar is 100 μm, with a first division of 10 μm.

cells with a cell body in the layer 1 (Stratum periventriculare, SPV).

For types I-XIII most of the properties are given in table 3, and these types are also quasi-schematically drawn to scale in figure 19, for which the values of table 3 were used. Since the cell types XIV and XV form heterogeneous populations, these two types are represented in figure 19 by a few characteristic specimens.

In the next paragraphs the cell types will be characterized and described in detail. Criteria used for the identification of the axons, which have sometimes rather unusual features, will be discussed in the DISCUSSION section on *Technical remarks*.

Type I cells are the most striking structures in Golgi-Kopsch sections. They have a bipolar soma in layer 5 and three separate dendritic trees: an apical tree in layer 7 and two basal trees, one in the lower part of layer 5 and the other in layer 3/4 (figs. 3a,b). The axon, arising from the basal dendritic trunk at the level of layer 4/5, arborizes and terminates in layer 3/4 (figs. 3c,d).

The soma position is mostly in the lower part of layer 5, but it can be more superficial, up to layer 6. Sometimes the soma might be called multipolar, when it gives rise to additional thin dendrites in layer 5 (figs. 3b,d). The dendritic tree in layer 7 is the most conspicuous one since it is composed of a large number of densely packed dendrites with many spines (fig. 24). Occasionally one or two of the dendritic trees are lacking (figs. 3d, 27), probably due to poor impregnation (see DISCUSSION section on *Technical remarks)*.

About half the cells show clearly the initial part of an axon (fig. 3a) but only for seven cells the axon, being very thin in layer 4, could be followed farther. It appeared to terminate in layer 3/4 (figs. 3c,d, 20-23) where the horizontal extension amounts to 50-200 μm, the same magnitude as the apical dendritic tree. At high magnification the axons have a very peculiar appearance and can be distinguished easily from the basal dendrites, although they bear incidentally spine-like protrusions (figs. 25, 26; see DISCUSSION section on *Technical remarks)*.

The above description is based on Golgi-Kopsch impregnations. In normal and diluted Golgi-Cox impregnations (METHODS), type I cells are found rarely. The 20 examples which yet could be selected in especially long GC + ey preparations showed the same characteristics as in Golgi-Kopsch sections, except that the sizes of the somata were about 25% smaller.

Type II cells are almost exclusively found in GC + ey or long GC + ey, conditions in which other cells except types I and XIV are seldom impregnated. The cell body can have a position from layers 3/4 to 4/5, and sends one or two, sometimes remarkably thin branches superficially, where they give rise to a rich plexus, especially for type IIa. Cells belong to *type IIa* when they have an apical dendritic tree in layer 7 (fig. 4a) and to *type IIb* when their apical dendritic tree lies in layer 6 (fig. 4d). Occa-

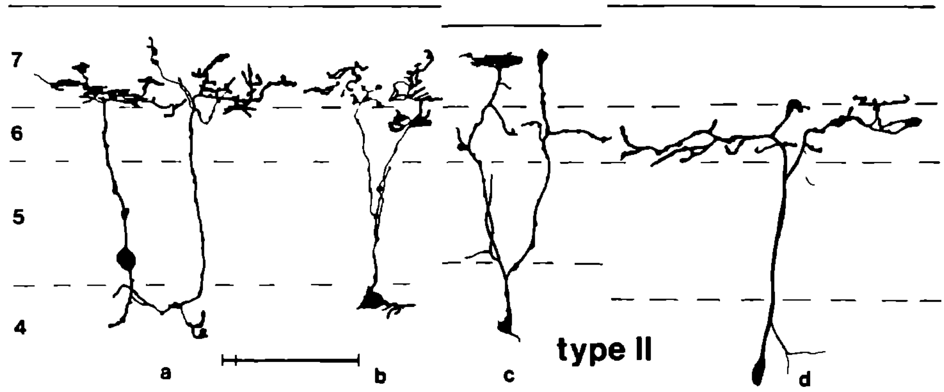

Fig 4 Figures a and b show type IIa cells figure d a type IIb cell The cell of figure c is transitional be tween types IIa and IIIb

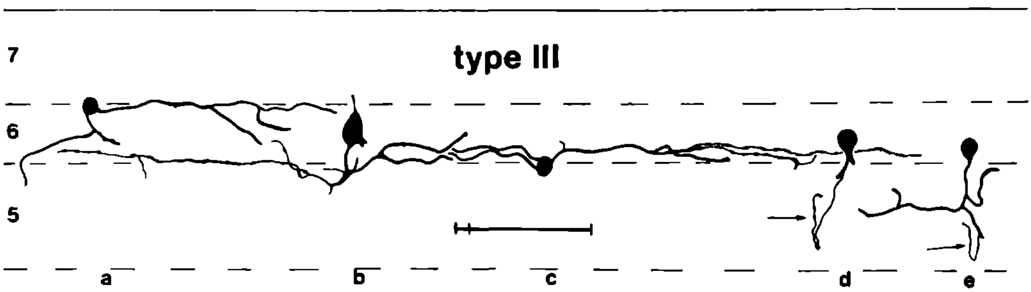

Fig 5 Type III cells

sionally intermediate cells, with dendrites in both layer 6 and 7 are observed (figs 4b,c)

Besides the apical dendritic tree, type IIa and b cells frequently have some tiny branches in layer 4/5 Most probably all branches are dendrites and an axon could never be found Some features, e g , the thickness of the dendrites, may be due to impregnation artefacts frequently observed in the superficial layers with the above mentioned impregnations

Type III cells are monostratified cells with a spherical soma in layer 6 and a single dendritic tree, mostly situated in layer 5/6 (figs 5, 27, 28) Mono-, bi- as well as multipolar somata are found Dendrites are not exclusively found in layer 5/6, but also slightly lower or higher, up to layer 6/7 (figs 5e and 5a, respectively) The axon arises from one of the dendrites, generally at a distance of 15-65 μm from the soma Often, only the first 20 40 μm of the axon was impregnated However, five specimens were found impregnated over a longer distance, and they seem to terminate in layer 5 (figs 5d,e)

Type IV cells have a single dendritic tree in or near layer 4/5 Based upon the horizontal dendritic extension they can be divided in large (IVa) and small cells (IVb)

The large *type IVa cells* have a cell body in layers 4 or 4/5, and a very broad dendritic tree (table 3, fig 6a) The axon arises from either a dendrite or a soma, and seems to remain within layer 4/5, although the axon was never impregnated over a long distance (figs 29, 30)

The small *type IVb cells* are frequently not well impregnated whereas no axon was found (figs 6b,c) In general appearance they resemble type III cells, except for their position

Type V cells are mono- or bipolar horizontal cells in layers 3 or 4 with one or two dendrites running parallel to the surface For so far as the axon has been impregnated, it arises from either the soma or a dendrite, and runs mostly parallel to the surface It does not seem to leave the tectum (figs 7, 31) In layers 3 and 4, long dendritic processes (> 1,000 μm) are found running parallel to the surface The cell bodies that give rise to these dendrites are in most cases not found, but type V cells seem the most likely candidates

Type VI This type of neuron has a bipolar

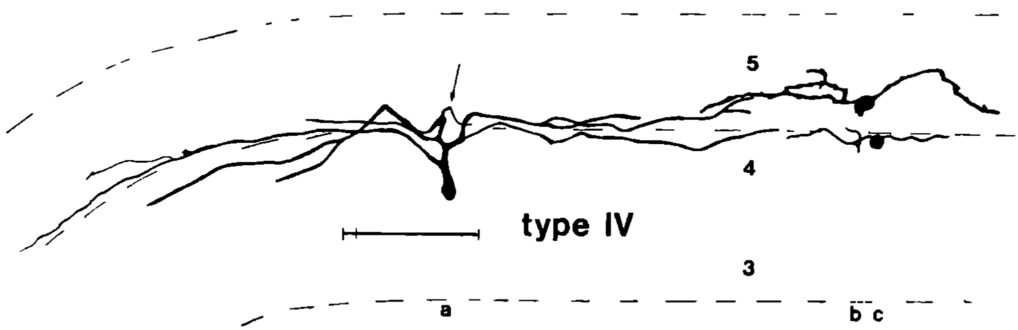

Fig 6 Figure a shows an example of type IVa and figures b and c examples of type IVb

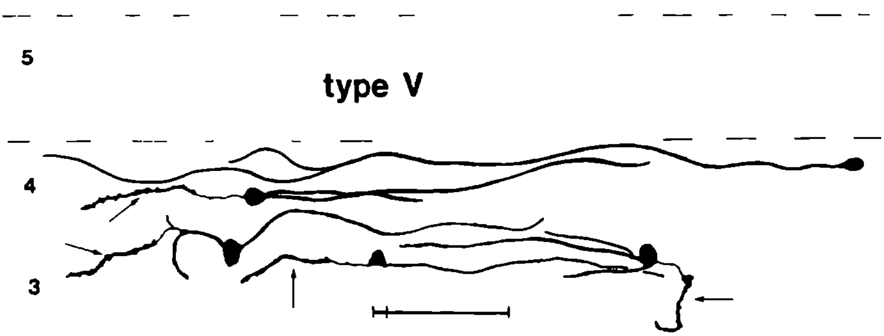

Fig 7 Type V cells

soma in layer 5 and dendrites terminating at two horizontal levels layer 6 and layer 4/5, or somewhat below this (fig 8 and 32)

The soma, which is sometimes tripolar, can be located in a broad region ranging from the lower part of layer 5 up to the lower part of layer 6 The axon originates from the basal dendritic tree in layer 4/5 and is directed downwards The impregnated part was always too short to follow the course of the axon Fortunately, three times in Golgi-Kopsch material the unimpregnated part of the axon appeared as a hollow thread, which was visible by its diffractive properties (figs 8a, 32, 33a) These unimpregnated parts traversed layers 4 and 3 and turned off horizontally in layer 2, which suggests that the axon leaves the tectum via this layer (see the DISCUSSION section on *Technical remarks)*

Type VII cells have a bi- or multipolar cell body, located in layer 4/5 and dendrites in two horizontal planes, a broad one in layer 4/5 and a relatively narrow one in layer 6 (fig 9) The axon arises from the soma or a dendrite near the soma with the initial part directed upwards Well impregnated axons arborize and seem to end in layer 5

Type VIII neurons are characterized by a dendritic tree in the lower part of layer 5 and another one in layer 3/4 The axon arises from the dendritic trunk in layer 4/5 (figs 10a,b, 34, 35)

The soma is basically bipolar and located in layer 4 However, when the soma is found in layer 3/4, then it is multipolar and when located in layer 3 monopolar (fig 10b) In one example the basal dendritic tree was not positioned in layer 3/4 but in the middle of layer 4, and in another example in layer 3 The axon of several cells was seen to arborize in the lower part of layer 5 (figs 10a,b, 34, 35) For one cell a peculiar collateral arborization was observed in deeper layers (fig 10c)

Type IX cells have a bi- or multipolar soma in layer 3/4 while their dendritic trees are both in layer 3/4 and in layer 4/5 or slightly higher The axon arises from the soma with the initial part directed upwards (fig 11) The axons seem not to leave the tectum, but to project upon structures in the neighbourhood of, or higher than the soma So, these neurons have the same characteristics as type VII cells, except that they are located one layer lower

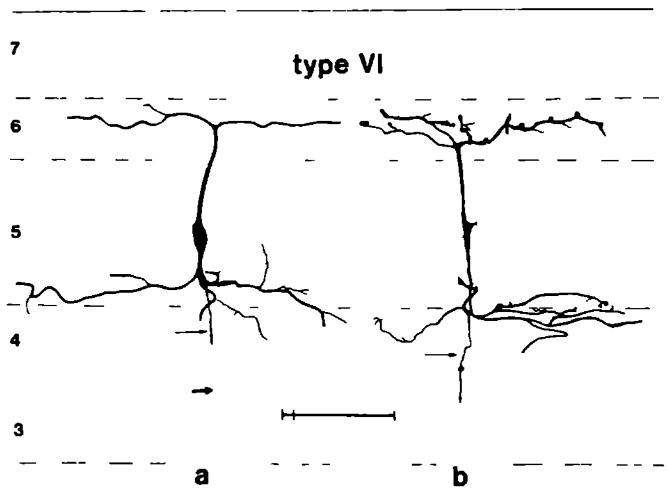

Fig 8 Type VI cells, thick arrow indicates the unimpregnated part of the axon, visible in Golgi Kopsch impregnations

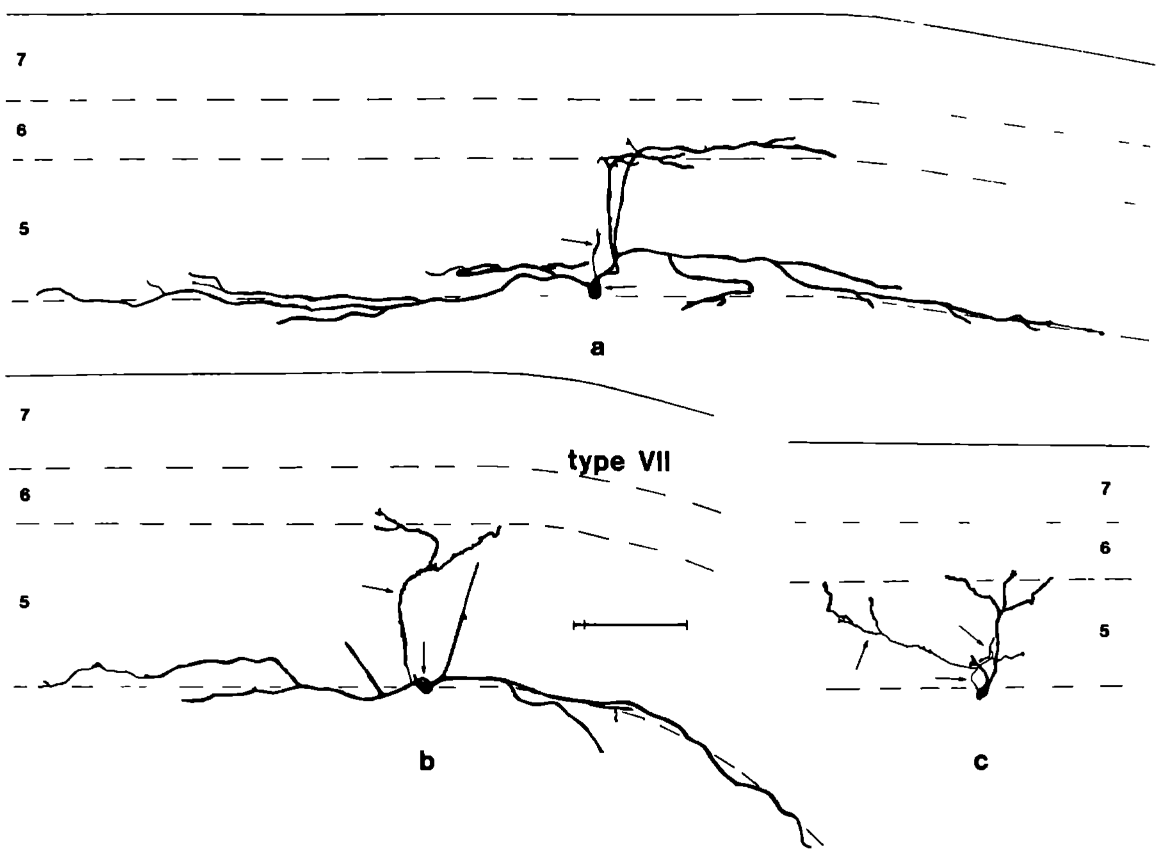

Fig 9 Type VII cells

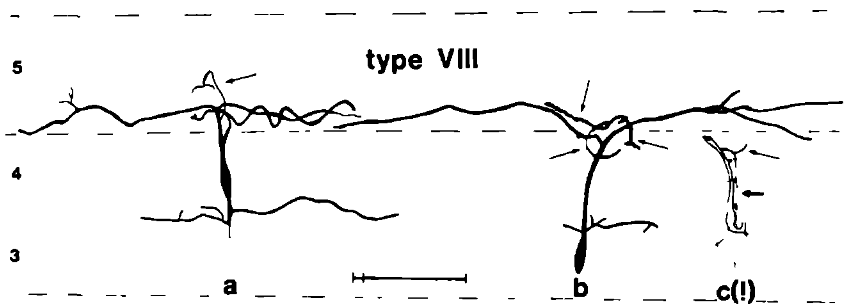

Fig. 10 Type VIII cells, thick arrow indicates a peculiar collateral. In figure c (!) only the axonal structures are completely drawn

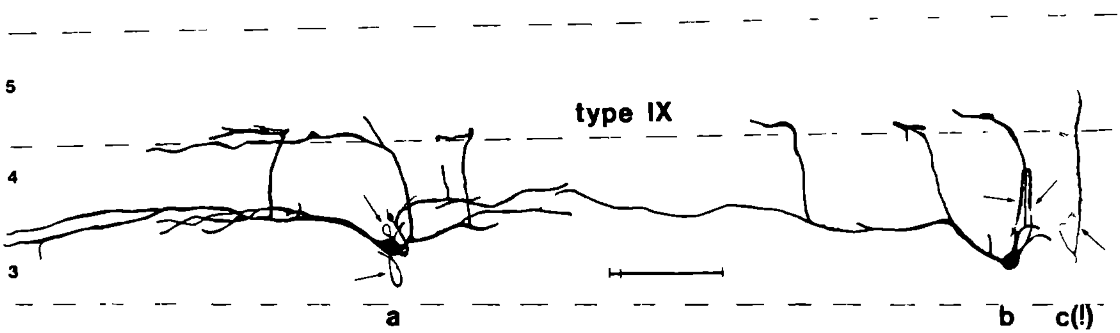

Fig. 11 Type IX cells. In figure c (!) only axonal structures are completely drawn.

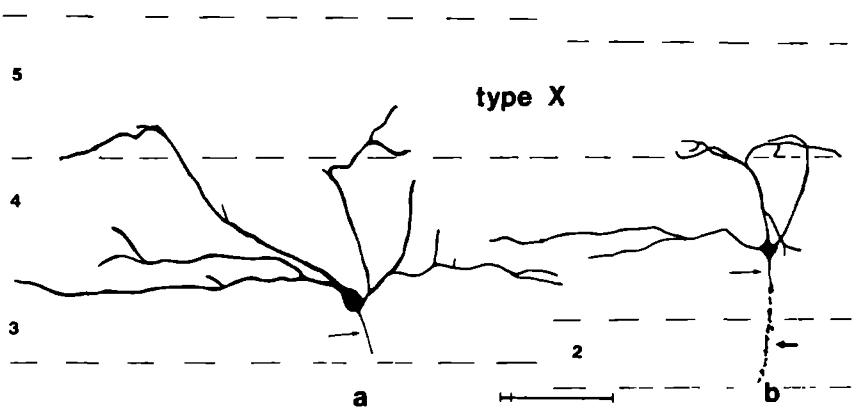

Fig. 12 Type X cells; thick arrow indicates the myelinated continuation of the axon as visible in ½ GC + ey.

Type X neurons have the same characteristics as type IX neurons, except that the axon directs downwards (figs. 12, 36). The axon probably leaves the tectum via layer 2, since for four cells, the myelinated continuation (Meek, '78) could be followed till this layer (figs. 12b, 36).

Type XI consists of cells with a bipolar cell body in layer 3, dendritic trees in layer 6 and 4/5 and a small arborizing structure in layer 2 (figs. 13a,b). The soma is sometimes tripolar, sending a dendritic branch to layers 3/4 or 2/3 (fig. 13b). No axon could be found, although the poor arborization in layer 2 sometimes has an axonal appearance. When the basal trunk is not impregnated this cell type roughly resembles type IIb.

Type XII cells have a bipolar soma in layers

3 or 4 and three dendritic trees, two situated apically and one basally The uppermost tree is located in layer 5 and protrudes into layer 6, the middle tree is found in layer 4 and/or in layer 4/5 and the basal tree extends in layer 2/3 The axon arises from the apical dendritic trunk in layer 4/5 between the two apical dendritic trees (figs 2, 14, 38)

The position of the soma varies substantially (figs 14a-c), from layer 4 down to layer 2, which makes the soma monopolar (fig 14c) The basal dendritic tree is sometimes not impregnated (fig 14d), as was also found for type XI neurons With the appropriate impregnation methods (½ or ¼ GC + ey) the initial part of the axon can be observed to be continued by the myelin sheath The axon leaves the tectum either directly (figs 14d, 38), or via layer 2 (fig 14e) The former was generally observed in the ventro-lateral part of the tectum

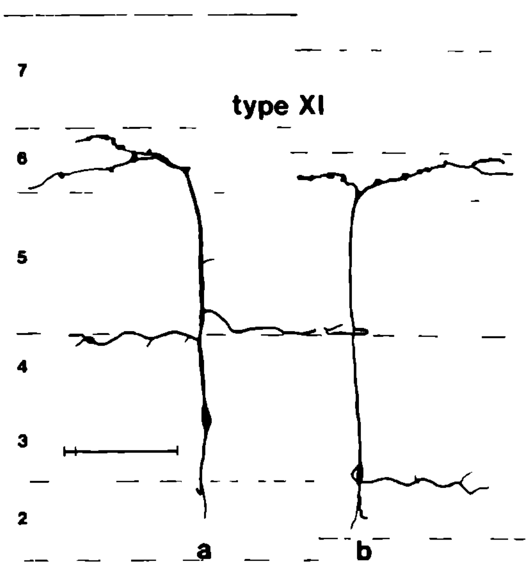

Fig 13 Type XI cells

Type XIII neurons have a multipolar cell body in layer 2 and a myelinated axon, arising from the soma or a dendrite near the soma and leaving the tectum Dendritic trees are found in four distinct regions layer 2, the upper part of layer 3, the lower part of layer 5 and layer 6 (figs 2, 15a,b, 37)

The cells can be subdivided into type $XIII_1$ and $XIII_2$ cells, based on the course of the axon which was observable by its myelin sheath Type $XIII_1$ has an axon initially directed straight upwards It courses to the upper part of layer 4 where it reverses direction and subsequently runs downwards parallel to the initial rising part (figs 15a-d, 39) This arciform axonal course is only observed for this subtype The axon leaves the tectum, either via layer 2 (fig 15d), or directly (fig 15c) The axons of type $XIII_2$ do not show the arciform course in layer 4 (fig 15e) and their initial part is in general oriented horizontally or

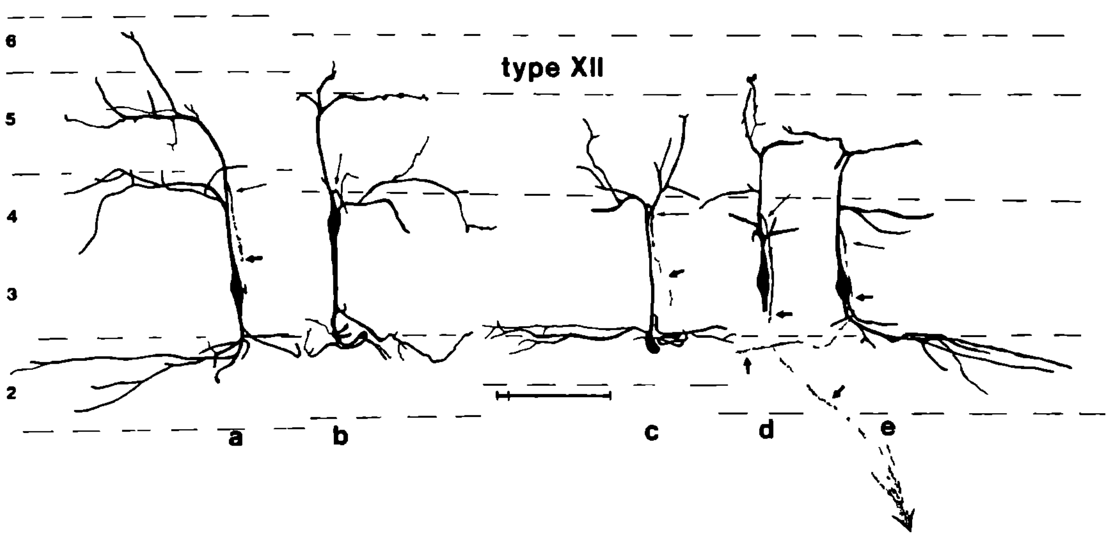

Fig 14 Type XII cells, thick arrows indicate the myelinated continuations of the axons as visible in ½ or ¼ GC + ey The axon in figure d joins other axons and the bundle could be followed further

obliquely. Some type XIII neurons could not be subdivided since their myelin sheath could not be followed and the first part of the axon was not oriented horizontally.

Other systematical differences between type XIII$_1$ and XIII$_2$ cells were not observed. For both types the shape and polarity of the cell body and the arborization pattern of the dendrites vary substantially. It is remarkable that in spite of these variations all multipolar cells in layer 2 have horizontally running dendrites at the same levels (compare figs 15a and 15b and see fig. 2). In table 3 no value is given for the diameter of the dendritic tree in layer 6 since these dendrites were generally poorly impregnated (see DISCUSSION section on *Technical remarks*). When all dendrites in layers 6, 5 and 3 are unimpregnated, these cells resemble a horizontal cell with dendrites in exclusively layer 2 (fig 15d). Their axonal course, however, allows for proper identification. In some, mostly oblique sections the lowest two basal dendritic trees, which can be clearly observed in sections perpendicular to the surface, would not be separated (fig 15c).

Type XIV neurons form the most numerous population of tectal cells, since most neurons with a soma in layer 1 (SPV) belong to this type. Although type XIV cells differ in the size of the soma, the position and extension of the dendrites and the course of the axon, they have such striking similarities that they are considered as a single population All neurons of this type share three characteristics.

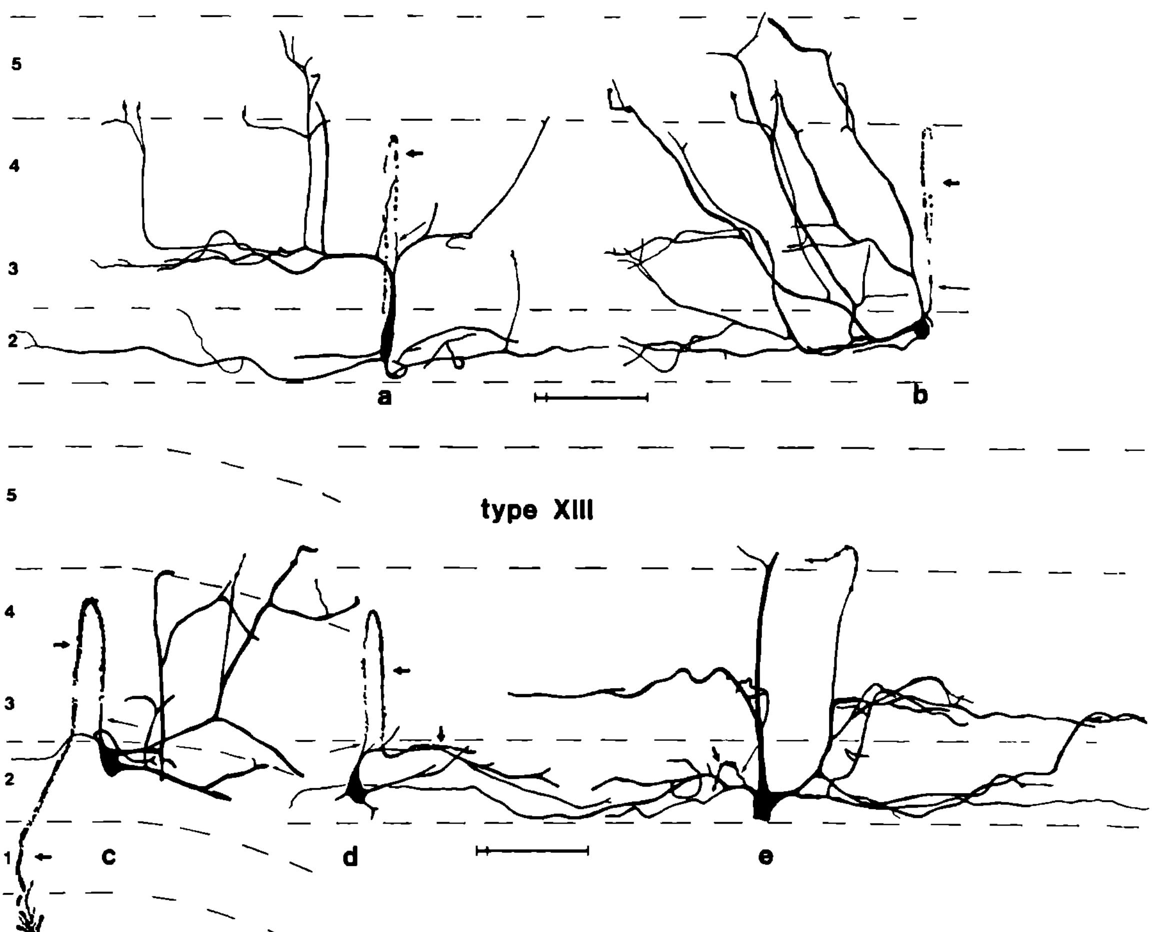

Fig 15 Figures a, b, c and d show examples of type XIII$_1$ and figure e, an example of type XIII$_2$, thick arrows indicate the myelinated continuations of the axons as visible in ½ or ¼ GC + ey The axon in figure 15c joins other axons and the bundle could be followed further

(1) There are always dendrites in one or both of the layers containing optic nerve fibers and/or terminals (layers 6 or 5). In addition, other dendritic trees may be present. These are frequently located in layers 4 or 3/4, seldom in layers 3, 2 and 1, and never in layer 7.

(2) The axon arises from the dendritic trunk or arborization in layer 4/5. A myelinated continuation of the initial part was

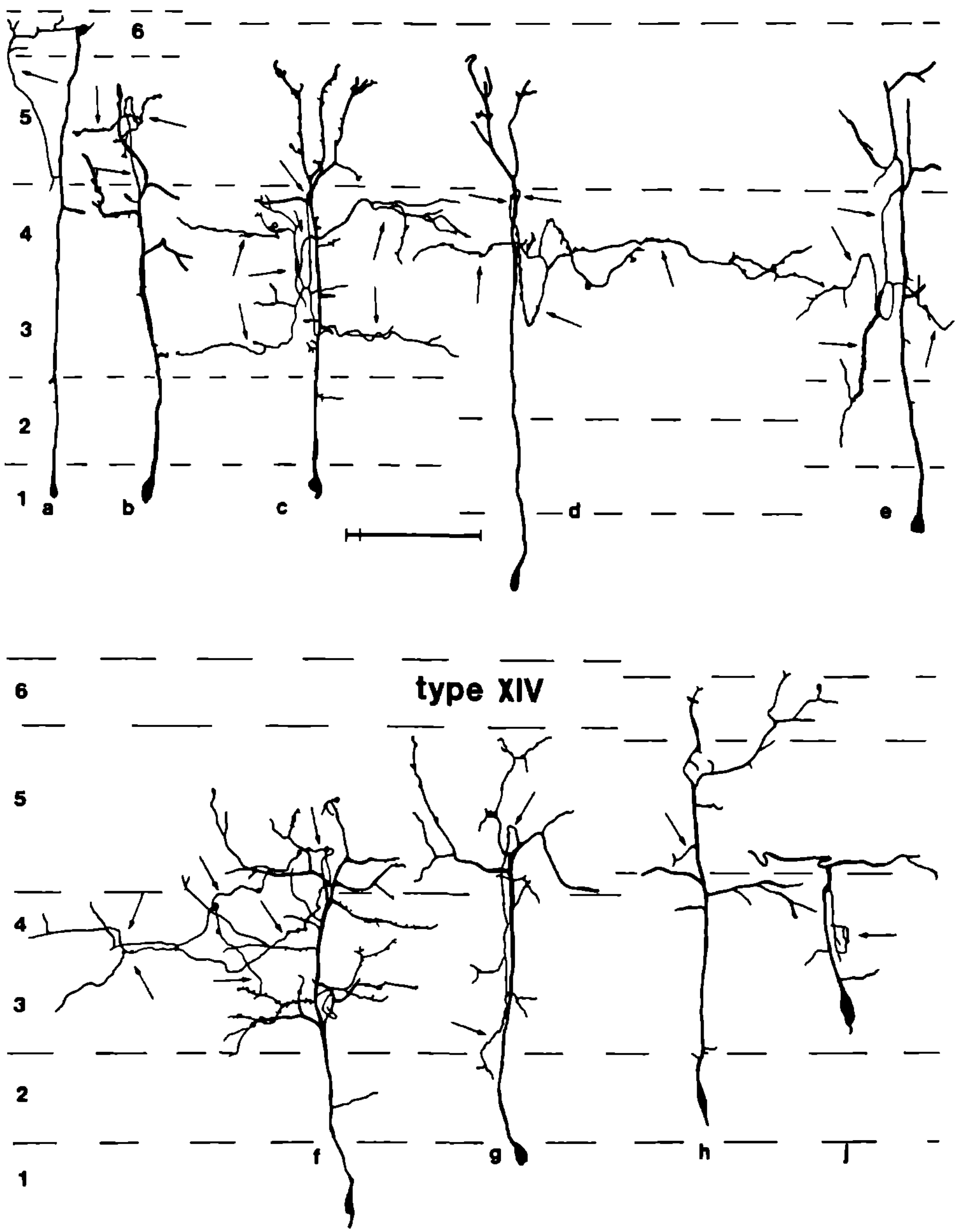

Fig. 16 Small type XIV cells. Figures f and g are impregnated with rapid-Golgi, figure j with Golgi-Kopsch and the remaining cells with ½ or ¼ GC + ey.

never observed When the axon could be followed over a sufficiently long distance, it appeared to terminate within the tectum Axonal branches spread from layer 2 to layer 6

(3) The soma is monopolar with a single apical dendritic process, projecting to more superficial layers Some cells have their soma not in layer 1 but in layer 2, or even 3 or 4

These three characteristics, which can vary substantially, will be described in detail in the next paragraphs

The dendrites The apical dendrites terminate in a broad region ranging from layer 4/5 to layer 6 as can be seen in figure 16 Some cells lack the dendrites in layers 2, 3 and 4 whereas other cells have very pronounced dendrites in layers 4 or 3/4 Although most cells do not show abundant spines, a few are quite spiny The horizontal extension of the apical dendritic tree is mostly between 50 and 100 μm, but a separate subtype with thicker dendrites extending over a much longer distance (more than 200 μm) can be distinguished (fig 17) These *"large" type XIV cells* have their apical dendritic tree mostly in layer 4/5 Besides they often show pronounced dendritic trees in deeper layers such as 3/4 or 2/3 (figs 17b,c) In other respects these cells have the same properties as the more common *"small" type XIV cells* (fig 16)

The axon This structure is not well impregnated in GC + ey and in Golgi Kopsch, where only rarely the initial part of the axon is found (figs 33e,f) In rapid Golgi and diluted Golgi Cox impregnations, however, the axon can be impregnated over a long distance (fig 16) The initial part may be directed upwards (figs 16b,f) or downwards (figs 16c,e), irrespective of the region of termination, which was always within the tectum To reach this region many axons have a looping course (figs 16d,g)

For the "small" cells several areas of termination can be distinguished The axon can project to layer 6 (fig 16a), possibly combined with some lower axonal branches It can also project to layer 5, whether or not combined with terminations in layer 4 (fig 16b), it can project exclusively to layer 4 and/or 3 (figs 16c,d,f, 40) and finally the axon may also run to the deep part of layer 3 or to layer 2, where it branches The last type frequently has collaterals in layer 3/4 (figs 16e,g, 41 43) In the first type of projection also dendrites are found in layer 6 (fig 16a) The other axonal projection types do not show a strict relationship with dendritic trees at a specific level

Also the axons of the "large" cells arborize and terminate in the tectum (fig 17a) However the axon is less easily impregnated and its arborization pattern differs from that of the "small" cells

The soma As mentioned most cells have their soma in layer 1 For the "small" cells the mean soma width and height is 8 and 10 μm respectively, for the "large" cells these values are 10 and 13 μm respectively Both types of cells sometimes have their cell body in layer 2, and "small" cells even in layers 3 or 4 (figs 16h,j, 17c,d) It is obvious from the examples shown, that these cells cannot be distin-

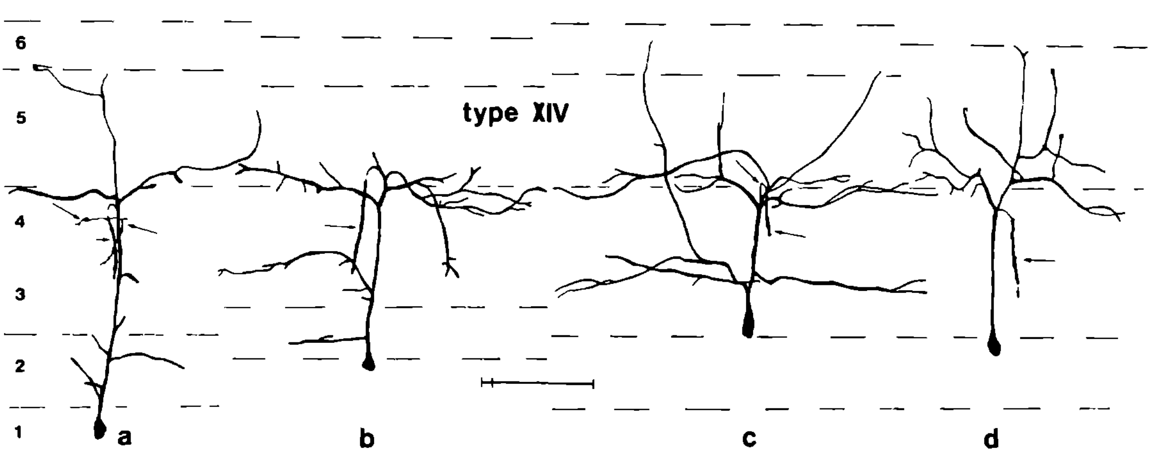

Fig 17 Large type XIV cells

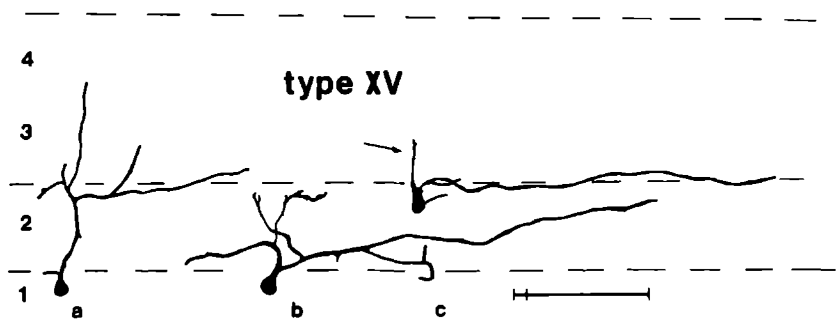

Fig 18 Type XV cells

guished from the deeper ones, except for their soma position

Type XV cells resemble type XIV cells in respect to their soma position in layer 1 (or sometimes 2) However, their dendrites do not project to the optic nerve layers 5 and/or 6, but terminate in deeper layers only, up to layer 4 (fig 18) Type XV cells occur less frequently than type XIV cells and are not thoroughly investigated They are mentioned solely to complete the picture of neurons with somata in layer 1

Unclassified tectal neurons About 10% of the cells found do not fit the above classification The properties of some of these cells can be defined unequivocally, but they were found only once or twice in the whole sample of about 575 cells drawn The other ones, usually positioned in layer 4, have obscure properties

The number of neurons per cell type

To estimate per tectal lobe the number of cells per type three procedures were applied

(a) Eight classes of cell bodies were distinguished based upon their shape and location (fig 19)

A globular cell bodies in layer 6 (belonging to type III cells),

B Fusiform, vertically oriented cell bodies in layers 5 and 6 (types I and VI),

C globular, roughly horizontally oriented cell bodies in or near layer 4/5 (types IV and VII),

D fusiform, vertically oriented cell bodies in layers 3 and 4 (types VIII, XI and XII),

E globular, roughly horizontally oriented cell bodies in or near layer 3/4 (types V, IX and X),

F multipolar, frequently rather large cell bodies in layer 2 (type XIII),

G cell bodies in layer 1 (types XIV and XV),

H the cell bodies of type XIV and XV cells that are higher positioned than layer 1, and the cell bodies of type II cells All of them are globular, roughly vertically oriented and may have the same position (layers 2-4/5)

Somata of class A-G were counted in sections of 10 μm obtained from four tecta (table 1) Class H cells could not be counted separately since they are easily confused with fragments of other cell bodies They were, however, included in the total number of neurons, in which possibly also some glial elements are included Besides the methodic and biological spread the countings are subject to errors in the identification of cell bodies, which can result in under or over estimations

Countings uncorrected for section thickness yield an upper limit of cells per class (table 1, column B) and countings corrected (Abercrombie, '46) a lower limit (column C) The latter correction is generally too large since cell bodies were only counted if a sufficient part of the cell body was available to recognize them

(b) It was assumed that within each soma class the composing cell types were equally present, unless the contrary appeared For class D this assumption is supported by the fact that types VIII, XI and XII are equally present in ½ GC + ey, since the impregnation probability in Golgi-Cox for different cell types with the same position seems equal (as shown by Pasternak and Woolsey '75 for mouse cortical neurons) The same holds for class E (types V, IX and X) and F (types $XIII_1$ and $XIII_2$) The cell types of class C (types IV and VII) were assumed to be equally present too, but without further support This, together with the results of table 1 yields a num-

TABLE 1

Mean number of tectal cells per section (A) and the extrapolated mean number of cells per half tectum uncorrected (B) and corrected after Abercrombie 46 (C)

	A				B	C
Fixation Staining	Bouin HE	Bouin LFB CFV	Formalin LFB CFV	Formalin LFB CFV	Average number per half tectum	Corrected number after Abercrombie
Soma class						
A (type III)	32±7 (10)	24±8 (4)	—	17±3 (4)	7 500	3 500
B (type I and VI)	73±6 (10)	81±8 (4)	48±5 (5)	56±18(4)	20 000	9 000
C (type IV and VII)	16±5 (10)	9±3 (4)	7±2 (5)	9±3 (4)	3 000	1 500
D (type VIII XI and XII)	13±3 (8)	16±2 (4)	18±3 (5)	17±3 (4)	5 000	2 500
E (type V IX and X)	23±5 (8)	15±3 (4)	12±2 (5)	13±5 (4)	5 000	2 000
F (type XIII)	8±2 (8)	9±3 (4)	8±2 (5)	7±1 (4)	2 500	1 000
G (type XIV and XV)	9 126± ?	6 479± ?	6 830± ?	—	2 000 000	1 000 000
Layer 5 + 6	142±9 (2)	139±8 (4)	113±12(5)	162±23(5)	40 000	20 000
Layer 3 + 4	97±8 (3)	89±5 (4)	88±22(5)	102±10(4)	30 000	15 000
Layer 2	77±11(3)	55±10(4)	93±30(5)	81±21(4)	25 000	10 000

H E Hematoxilin Eosin LFG CFV Luxol fast blue Cresyl fast violet (Kluver and Barrera 53) For the definition of the soma classes see text

ber of about 750 (column C) to 1,500 (column B) for cell types IV, V and VII-XII A safer estimate is 500-2,000, when errors in the identification and the fact that 10% of the cells were not classified are considered The number of type XIII cells is also 500-2,000, since for these large cells the value corrected for section thickness seems more likely than the uncorrected value The number of type III cells is 2,500-10,000

However, the cell types of class B, types I and VI, seem to be not equally present since in Golgi Kopsch type I is much more frequently impregnated than type VI, and for reasons presented under (c) Further, nearly all cell bodies of class G belong to type XIV cells, together with several class H cells, so that the number of type XIV cells becomes 1,000,000-2,000,000, whereas the number of the remaining type XV cells remains unknown

(c) The number of arciform and vertically running myelin sheaths in layers 3 and/or 4 was determined at ca 270 and 1,590 respectively in one tectal lobe treated with ½ GC + ey Arciform axons belong to type $XIII_1$ and since 60% of the impregnated type $XIII_1$ cells showed the arciform part of their axon in the sections considered, the total number of type $XIII_1$ cells should be ca 450 per tectal lobe The same number of type $XIII_2$ neurons may be expected (see under [b]) and the total number of 900 type XIII cells is in agreement with the estimates obtained by the first two procedures

Assuming that also 60% of the vertically running myelin sheaths, belonging to types VI, X and XII, was counted, their total number becomes ca 2,700 The number of types X and XII was already estimated under (b), and substraction of these values gives for type VI cells an upper limit of about 2,000 cells This implies that the remaining cell bodies of class B, 5,000-20,000, should belong to type I cells

Summarizing, these results yield an estimate of 500 2,000 neurons per tectal lobe for types IV-XIII The number of some other cell types were estimated as type I, 5,000 20,000, type III, 2,500 10,000, type XIV, 1-2,000,000 For types II and XV no estimate can be given

DISCUSSION

Technical remarks

Individual cells sometimes do not show all the dendritic ramifications a cell type has This is often caused by incomplete impregnation, as is indicated by the following three phenomena The first one is the occurrence of thick, blind ending dendritic trunks This is especially found for the tree in layer 3/4 of type I (fig 27), the tree in layer 6 of type VII (fig 9b), the tree in layer 2/3 of type XII (figs 14d, 38) and several trees of type XIII (fig 15c) A second indication of the incomplete impregnation is the dependence between the ratio of complete/incomplete cells and the impregnation procedure For instance, in ¼ GC + ey only 50% of the type XII cells showed the dendritic tree in layer 2/3, whereas in ½ GC + ey all cells had this tree, and in one Golgi Kopsch impregnated tectum only a small proportion of the type I cells had a dendritic tree in layer 3/4, but in another one it was clearly present in about half the number of cells. Thirdly, the common lack of impregnated axons demonstrates convincingly that whole parts of neurons can be missed.

On the other hand, the possibility cannot be excluded that a minor proportion of the cells really does not have a specific dendritic tree. For instance, the dendritic trunk of type I, that gives rise to the dendrites in layer 3/4, can vary substantially in thickness (figs. 20-22) and one could imagine that the cell of figure 23 does not have this dendritic tree at all.

There are no absolute criteria for the morphological distinction between axons and dendrites. However, the character and course of the myelinated axons of types X, XII, $XIII_1$ and $XIII_2$ cells could be established by means of the myelin impregnation in diluted Golgi-Cox fluids (½ GC + ey or ¼ GC + ey; Meek, '78).

Type VI cells are so superficially situated that their axons had to be studied in Golgi-Kopsch impregnations. Here the course of the unimpregnated continuation of these axons indicated that they leave the tectum (fig. 32). The efferent nature of the axons of type VI cells is also supported by the observation that their features resemble strongly those of the efferent axons of Golgi-Kopsch impregnated type X and type XII cells (figs. 33a-c). In contrast, the initial parts of axons that arborize within the tectum (e.g., those of types I and XIV, figs. 33d-f), look different and thinner in Golgi-Kopsch.

General criteria used to distinguish unmyelinated axons from dendrites are the smaller diameter, the orthogonal branching and the lack of spines. The axons of the small type XIV cells fulfill all these criteria when impregnated in rapid Golgi solutions (figs. 16f,g, 40). Once identified, the processes appear to have such a typical site of origin and initial course, that solely upon these two characteristics these can be distinguished as axons, even in diluted Golgi-Cox impregnations (figs. 16a-e, 41-43). In the latter impregnations the axons may show typical thicker parts, not observed for dendrites (figs. 16e, 42, 43). Although these thicker parts resemble to some extent the myelin impregnation, they can be distinguished since (a) the axon is also impregnated (figs. 42, 43, 45), (b) the thicker parts begin and end irregularly along the axon (fig. 42) and (c) these substructures are not globular but extend over some length around the axon (fig. 45). In diluted Golgi-cox impregnations these thicker parts can be used as an additional criterion to identify unmyelinated axons, not only for the small type XIV cells but also the type V (figs. 7, 31), type VII (fig. 9), type VIII (figs. 10, 35), type IX (fig. 11) and the large type XIV cells (fig. 17). However, not all axons of these cell types have thicker parts in diluted Golgi-Cox impregnations (figs. 7, 9, 10, 11, 34, 41).

Axons of type I and III cells could be studied only in Golgi-Kopsch impregnations, since these cells, just as type VI, are not impregnated in diluted Golgi-Cox. The axons of type I could be distinguished quite well from the basal dendritic tree, since they are initially much thinner, branch orthogonally and have typical "beaded" appearance, whereas the basal dendrites are initially thicker and have many spines (figs. 25, 26). Sometimes, however, the picture is confusing since the axons also can have spine-like protrusions, arising from the "beads." These substructures are probably not spines but short collaterals or filopodia since they are larger and their stalks longer than spines (figs. 25, 26), and since these stalks can continue beyond the thickening.

In the rare cases that type III axons are impregnated over a large distance, they resemble the axons of types I and XIV in respect to the initial thin part and the somewhat beaded structure. Since they furthermore sometimes have a looping course back to a more superficial level, they are likely to terminate in the tectum. The "beaded" appearance of type I and III axons (and also of type XIV axons) can unfortunately not generally be used for axon identification, since this feature is also observed in some dendrites (e.g., figs. 13a,b [type XI], 15e [type XIII], 40 [type XIV]).

Evaluation of Golgi studies on teleost tecta

The comparison of the present results with the findings of Leghissa ('55, mainly goldfish), Vanegas et al. ('74b, *Eugerres plumieri),* Ramon Cajal (1899, *Barbus fluviatilis)* and Bathelt ('70, *Salmo irideus)* is summarized in table 2.

Slight differences between the present study and Leghissa's, both on goldfish tectum, may be due to differences in impregnation techniques, in the age of the animals used and the inclusion of other teleost species by Leghissa. Furthermore, Leghissa was mainly interested in the general cytoarchitecture and

TABLE 2

Comparison of teleostean tectal cell types described by several authors

Present results Type	Leghissa '55 (goldfish) Type	Vanegas et al '74 (*Eugerres plumieri*) Type	Soma size	Extension	Ramon Cajal 1899[1] (*Barbus fluviatilis*) Figure	Bathelt, '70 (*Salmo irideus*) Type
I	m, cellule fusiformi di conspicuo volume	Large pyramidal neurons	8 12 μm	400 600 μm	3 D,G,H	Bipolare bidendritische Neurone in Stratum 5
IIa	o? picole neuroni dell typo piramidale; i? piccola cellula pira midale	Small pyriform neurons	5 8 μm		–	–
IIb	i? piccola cellula pira midale; o? picole neuroni dell typo piramidale					
III	n? piccole cellule piri-formi marginali	Neurons with a cell body in the stratum opticum	10 15 μm	400 600 μm	3 F	Marginal Neuronen?
IVa	l, cellule multipolari orizzontali	Horizontal cells	6 12 μm	100 μm	3 E	Multipolare Zellen in Stratum 5
IVb	l, cellule multipolari orizzontali					
V	–	Horizontal neurons		200 μm	3 d	Multipolare Neurone in Stratum 3
VI	only in figures	Small bipolar neurons	6 μm		3 I	–
VII	l, cellule multipolari orrizontali	Horizontal cells	6-12 μm	100 μm	–	Multipolare Zellen in Stratum 5
VIII	–	Small bipolar neurons?			–	–
IX	–	Small multipolar neurons?			–	–
X	–	Small multipolar neurons?			3 b	–
XI	only in figures (h?)	–			–	–
XII	h, cellula fusiforme	Fusiform neurons	6 10 μm	600 μm	1 E, 2 C	Bipolare bidentrische Neurone in Stratum 3
XIII$_1$, XIII$_2$	c, conspicui neuroni multi-polari	Large multipolar neurons	15 30 μm	600 μm	2 A,B	Multipolare Neurone
XIV, small (soma in layer 1)	a, cellula piriforme periventricolare	Tightly packed pyriform neurons	6 8 μm		1 B,C,D,F	Neurones piriformi
XIV, large (soma in layer 1)	a? cellula piriforme periventricolare	idem?			idem?	idem?
XIV, small or large (soma in layer 2 or 3)	e, cellule piramidali di medio volume; f, cellule piramidale; g, grosse cellule pira midali	Large pyriform neurons	10 15 μm		–	Bipolare axo dendri tische Neurone? Monopolare Neurone
XV (soma in layer 1)	b, neuroni multipolari periventricolari	–			3, below	–
XV (soma in layer 2)	d, picoli neuroni multi-polari	–			–	–

The quantitative data of Vanegas et al ('74) are included for comparison with table 3

[1] Figures 1 2 and 3 of Ramon Cajal (1899) correspond to figures 144 145 and 146 respectively of Ramon y Cajal ('11)

layer composition of fish tectum, whereas we are interested in a more quantitative morphological description of neurons in the goldfish tectum.

Some serious differences and even discrepancies also can be noticed. Firstly, Leghissa described cell types that were not observed in the present study (his type o and n) and vice versa (our types IIa, III, IV, V, VI, VII, VIII, IX, X and XI). However, Leghissa's type o resembles our type IIa, although type o axons run to layer 7, whereas we never observed an axon but found dendritic structures in layer 7 that were not described by Leghissa. Further, type n and type III share their soma position in layer 6, but in contrast to our neurons with somata in layer 6, type n has dendrites in layer 3/4 and an axon running to layer 7. Type IV, V and VII cells may all be included in Leghissa's type l. The axons of some of these type l cells project to the basal dendrite of type m (=I) cells. They are most likely our type IV cells, since types V and VII project somewhere else. Types VIII, IX and X are represented only in Leghissa's figures and not mentioned in his text. This holds also for type XI, which is probably included in Leghissa's type h.

Type I agrees with Leghissa's type m, except for the axon. For type m cells the axon is myelinated and leaves the tectum, whereas for type I cells the axon is unmyelinated and terminates in layer 3/4. The most plausible explanation for this discrepancy is that the axons Leghissa describes for type m cells are, in fact, the efferent axons of our type VI cells, since Leghissa did not separate this type from his type m and since type VI cells are confused easily with type I cells in case the apical dendritic tree is not clearly visible. The observation that the site of axon origine for type m cells in Leghissa's figure 5 resembles more the axon origin of type I than of type VI may be due to the fact that this figure is a reconstruction for which several teleost species were used. This in contrast to Leghissa's figure 6, based solely on goldfish, where the axons of type m totally agree with the axons of type VI. Consequently, we conclude that the axons of goldfish type I tectal neurons terminate in layer 3/4, just as Vanegas et al. ('74b) found in *Eugerres plumieri* and Ramon Cajal (1899) in the barbel.

Leghissa's type h consists mainly of our type XII cells, as is especially obvious from the axonal properties (myelination, recurrent course). However, Leghissa did not describe dendrites in layers 4 or 4/5, and only a small basal dendritic tree. Cells with an axon arising at the basal dendritic trunk as drawn by Leghissa were not found in our preparations; these cells might be our type XI cells, whose axons we did not observe. The large efferent type XIII cells are less pronounced in Leghissa's description. They are presented as type c, and sometimes as unclassified pyramidal cells with an arciform efferent axon. The axonal properties form also here a base for comparison.

Our type XIV coincides with Leghissa's types a, e, f and g. However, Leghissa describes mostly axons arising basally from the soma and leaving the tectum, whereas we found almost exclusively axons arising at the dendritic trunk or tree at the level of layer 4/5, arborizing and terminating within the tectum. Only in Golgi-Kopsch preparations we observed two cells with a basal protrusion that could be axonal in a sample of about 2,000 cells that were searched for this characteristic, and only for our last axonal projection type we cannot exclude that the lowest axonal branch in layer 2 leaves the tectum. Anyway, Leghissa missed the structures that we describe as axons and it is doubtful whether the structures he describes are the axons of especially his type a. Also Vanegas et al. ('74) did not find basally arising axons in *E. plumieri*, even with electron microscopy (Laufer and Vanegas, '74), as was also the case for the related carp tectum (Ito, '70). Moreover, Ramon Cajal (1899) and Vanegas et al. ('74b) found axons or axon origins strongly resembling ours, and so it seems reasonable to conclude that the majority of type XIV cells are interneurons.

There is a strong similarity between the present study and that of Vanegas et al. ('74b). In the tecta of both studies roughly the same cell types are recognized. Our types IV and VII were not especially classified by Vanegas et al., who showed, on the other hand, quite a variability in horizontal cells of layer 5, and also type XI is not described, but the other cell types are identical (types I, II, III, XII, XIV) or nearly the same (types VI, VIII, IX, X, XIII). Some quantitative differences might be due to species differences, like the horizontal dendritic extension of type I and III cells (*E. plumieri*, both 400-600 μm; goldfish 120 ± 50 and 130 ± 80 μm respectively). All in all, both tecta seem basically similar, in spite that different teleost species were used, one living in brackish lagoons and the other domesticated The same holds in relation to the barbel, *Barbus fluvius,* studied by Ramon Cajal (1899) and cited by Ramon y Cajal ('11) The cell of figure 3C of Ramon Cajal (1899) is especially interesting It is a type XIV cell with an axon that corresponds to our last axonal projection type The basal collateral, however, apparently leaves the tectum This could indicate that not all type XIV cells are interneurons

The Golgi study of Bathelt ('70) on the tectum of the trout *(Salmo irideus)* is less extensive, but the cells described can easily be recognized and compared with our cells The data of Bathelt ('70) on the axonal properties of type I, III, and XIV cells agree with Leghissa's findings, which are however, as discussed above, not in line with our results

Tectal cytoarchitecture

The structure of the tectum suggests that each layer or sublayer represents a specific stage in signal processing, since dendritic and axonal terminations are confined to specific levels Therefore we have not classified the cells primarily based upon soma characteristics (multipolars, fusiforms, pyriforms, etc) but according to dendritic and axonal properties To group the cell types dendritic properties were used (fig 19) In this way we distinguish *monostratified* cells (with dendrites at a single level, group 2 in fig 19), *bistratified* cells (with dendrites at two separate levels, group 3), *multistratified* cells (group 4) and *non stratified* cells (group 5) This terminology is adopted from Ramon y Cajal (1893) Two types of cells (type I and II) are regarded as a separate group (group 1 in fig 19) since they have dendrites in layer 7 This layer is only present in fish (cf Szekely, '73) and consists of unmyelinated axons of unknown origin (Buttler and Ebbesson, '74) Many synapses between these axons and the dendrites of type I cells have been found in *E plumieri* (Laufer and Vanegas, '74)

The multistratified group 4 cells (types XI XIII) are for the major part efferent and it is remarkable that only these cells have large dendritic trees in deeper tectal layers (layers 2 and/or 3) Consequently they process not only visual, but also other signals, which do not originate inside the tectum since no tectal axon terminates specifically in layers 2 or 3 The origin of these axons can be diverse, since in the layers deeper than the optic nerve layers 5 and 6, nerve bundles terminate from the contralateral tectum (Ebbesson and Vanegas, '76), the telencephalon (Vanegas and Ebbesson, '76), the nucleus isthmi, the thalamus, the cerebellum and the spinal cord (Ariens Kappers, '21, Kirsche and Kirsche, '61, Schnitzlein, '64)

The non stratified cells, especially type XIV, have all kinds of combinations in dendritic and axonal termination regions Moreover, they are very numerous So, the cytoarchitecture of goldfish tectum appears to consist of a fairly strictly structured frame, formed by type I XIII cells, intermingled with the variable type XIV cells

Besides the vertical position of dendritic trees also their horizontal extension should be considered In the layers where the optic nerve fibers terminate (4/5 6) the diameters of the dendritic trees range in mean value from 60-600 μm (table 3), corresponding to 2 20° of visual angle as calculated from the retinotopic projection (Jacobson and Gaze, '64), which compares with the receptive field sizes of tectal neurons (Schellart and Spekreijse, '76) The dendritic field extension together with the cell density gives the amount of overlap of a dendritic field For the very numerous type XIV cells this overlap ratio is 325/1, when the diameter of their dendritic fields is set at 75 μm, their number at 1,500,000 and the tectal surface at 20 mm^2 For any other type a much lower overlap ratio is found since their number is much lower, and for the most frequently found field diameter of 100 200 μm (table 3) of types with 500 2,000 cells there is even no overlap at all

From figure 19 it can be seen that each tectal layer is characterized by its own population of cell bodies, dendrites and axons, which can be very complex (e g , for layer 4/5) Also tectal afferents have to be included Roughly three regions of afferents can be distinguished (1) the marginal axons in layer 7, (2) the optic layers (6, 5 and 4/5) and (3) the deeper non optic layers In the latter, axons from various parts of the brain terminate as discussed above, while also the axons of type I cells terminate here, providing for indirect input from the marginal axons None of the mono- and bi-stratified interneurons (types III V and VII IX) have axon terminations in these non-optic layers

The axons of most tectal cells originate just in between the optic and the non optic layers in layers 4/5 or 4 (types I, IV, VI, VII, VIII, XII

TABLE 3

Quantitative properties of the cell types

Group	Type	Soma				Dendrites			Axon		
		Position (layer)	Basical polarity	Width (μm)	Height (μm)	Position (layer)	Extension (μm)	Maximal extension (μm)	Origin (layer, structure)	Arborizing or efferent	Layer of termination
1. Dendrites in layer 7	I (84)	5	bi-	13± 2(60)	19± 4(60)	7	120± 50(68)	250	4/5, dendrite	arborizing	3/4
						5	60± 30(59)	150			
						3/4	40± 25(32)	100			
	IIa (10)	4/5(3/4)	bi- (mono-)	12± 3(10)	11± 3(10)	7	100± 50(10)	175	?	?	?
						4/5	60± 10(5)	75			
	IIb (8)	3/4(4/5)	mono- (bi-)	9± 2(8)	13± 2(8)	6(7)	130± 80(7)	250	?	?	?
						4/5	30± 20(3)	50			
2. Mono-stratified	III (41)	6	variable	13± 2(34)	13± 2(34)	5/6 (6/7,5)	130± 80(33)	300	5/6, dendrite	arborizing	5
	IVa (9)	4(4/5)	variable	11± 4(6)	16± 4(6)	4/5	410± 250(6)	700	4/5, soma or dendrite	arborizing	4/5?
	IVb (10)	4/5, 5	variable			4/5(5)			?	?	?
	V (11)	3 or 4	bi- mono-	13± 4(10)	12± 3(10)	3 or 4	230± 100(11)	500	3 or 4, soma or dendrite	arborizing	3 or 4
3. Bi-stratified	VI (18)	5	bi-	10± 2(18)	18± 4(18)	6	160± 50(14)	250	4/5, dendrite	efferent	—
						4/5	160± 70(13)	250			
	VII (10)	4/5	multi-	12± 4(7)	12± 3(7)	6	100± 50(5)	250	4/5, soma or dendrite	arborizing	5
						4/5	600± 300(6)	900			
	VIII (14)	4(3)	bi-	9± 1(11)	19± 5(11)	4/5	240± 100(11)	350	4/5, dendrite	arborizing	4/5
						3/4	90± 40(11)	200			
	IX (12)	3/4	multi-	12± 4(10)	14± 3(10)	4/5	220± 110(9)	400	3/4, soma	arborizing	4/5
						3/4	270± 210(9)	600			
	X (14)	3/4	multi-	13± 4(14)	14± 3(14)	4/5	150± 90(10)	250	3/4, soma	efferent	—
						3/4	200± 120(11)	400			
4. Multi-stratified	XI (10)	3	bi-	7± 2(10)	18± 3(10)	6	130± 70(10)	250	?	?	?
						4/5	130± 70(10)	200			
						2	15± 6(8)	25			
	XII (30)	3 or 4	bi-	11± 2(28)	20± 4(28)	5(6)	90± 50(24)	150	4/5, dendrite	efferent	—
						4(4/5)	120± 70(26)	250			
						2/3	160± 60(23)	250			
	$XIII_1$ (20)	2	multi-	13± 3(18)	25± 5(18)	6	?		2, soma	efferent (with an acriform course in layer 4)	—
						4/5	140± 80(12)	250			
						3 2	300± 90(18)	500			
	$XIII_2$ (12)	2	multi-	14± 3(11)	22± 7(11)	6	?		2, soma	efferent	—
						4/5	150± 110(9)	300			
						3	420± 60(6)	600			
						2	380± 240(7)	700			
5.	XIV (186)	See text.									
	XV (33)	See text									

The fifth, sixth and eighth column give the mean value and standard deviation In parantheses the number of structures studied is given For definition of the groups (first column) see the DISCUSSION on functional significance Further comments are given in the RESULTS section

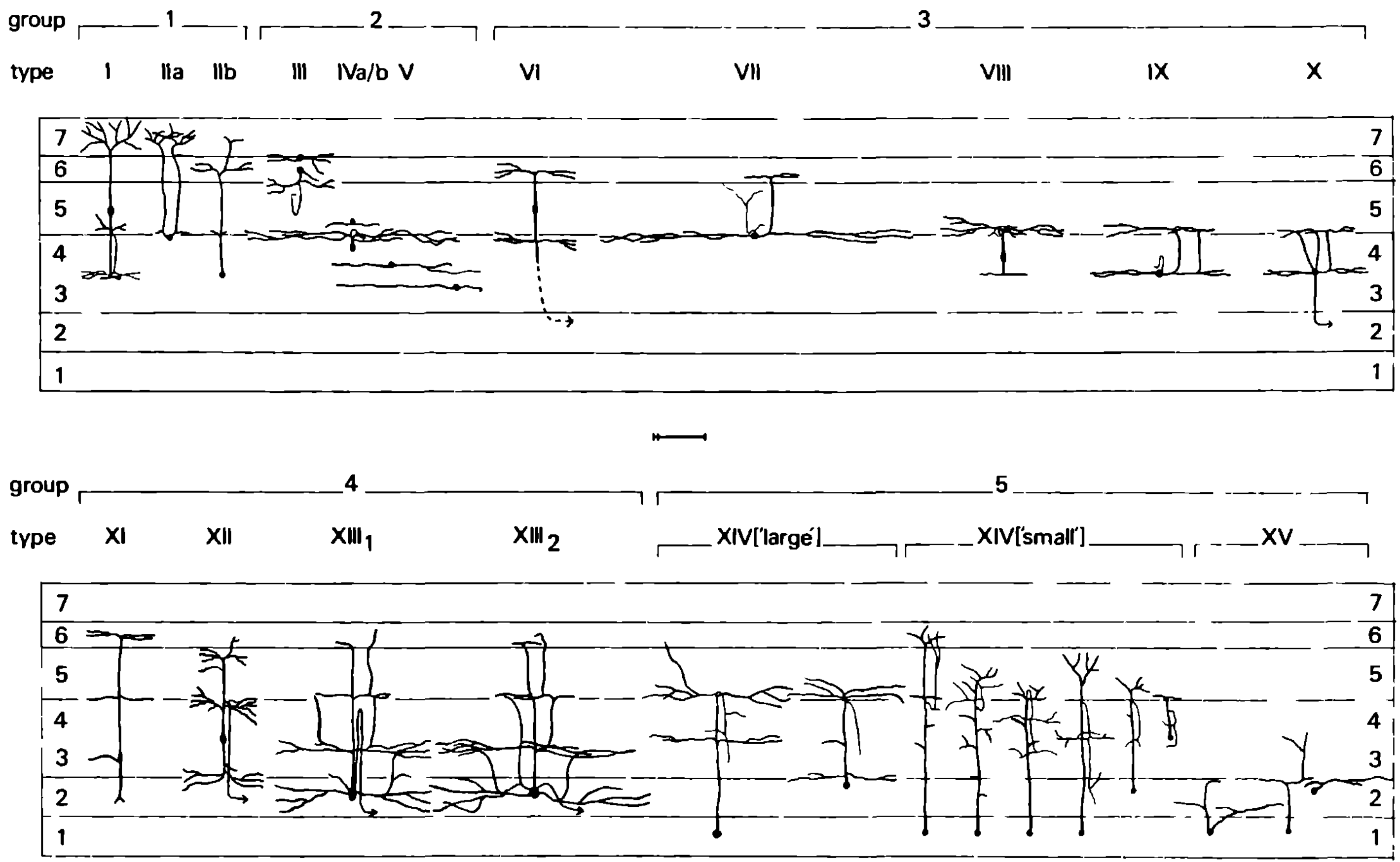

Fig 19 Schematic representation of the 15 tectal cell types described For types I XIII$_1$ the soma size and the horizontal dendritic extension are drawn to scale, according to the mean values of table 3 For types XIV and XV some typical neurons are drawn.

and XIV) or in layers 4 or 3/4 (types IX and X). The axons of type $XIII_1$, not arising at this level, pass also through this area before leaving the tectum. Since also the origin of the tectal evoked response (TER) is found at the level of layer 4/5 (Sutterlin and Prosser, '70; Vanegas et al., '71), all these types may contribute to the TER. For several types the axon does not arise from the cell body but from a dendrite (types I, III, VI, VIII, XII and XIV). This might suggest that the soma of these neurons has no direct function in the electrical activity. This holds especially for type XIV, where the distance between the soma and axon origin is about 250 μm. However, the studies of Skrzipek ('69) and Kroker ('73) reveal that their cell bodies play some role in signal processing, since their RNA- and protein synthesis is enhanced after visual stimulation.

In comparison to the 220,000 retinal ganglion cells which feed into the tectum (derived from Schellart, '73), for all types except type XIV a severe reduction in number is observed. Also the total number of myelinated tectal efferents, i.e., the total of type VI, X, XII and XIII cells, estimated at 2,000-8,000, is much lower. The number of unmyelinated tectal efferents is not clear. In our study no unmyelinated axon or collateral could be found that left the tectum or followed a course in layer 2, although axons were frequently well impregnated, especially for type XIV. However, Ramon Cajal (1899) described them for periventricular cells in the barbel, as mentioned above.

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PLATE 1

EXPLANATION OF FIGURES

20-23 Basal part of four type I neurons, all showing an impregnated axon (arrow) Notice the difference in thickness and development of each basal dendritic trunk and tree. Golgi-Kopsch. Calibration bar: 10 μm

24 Detail of the thick, spiny apical dendrites in layer 7 of type I neurons. Golgi-Kopsch. Calibration bar: 5 μm.

25, 26 Details of figures 21 and 20 respectively, which show the basal dendrites with real spines and the axonal branches, with "beads" and "spinelike protrusions" Golgi-Kopsch Calibration bar: 5 μm.

27 Low magnification photograph of a Golgi-Kopsch preparation, showing two type I cells and one type III cell Calibration bar: 100 μm.

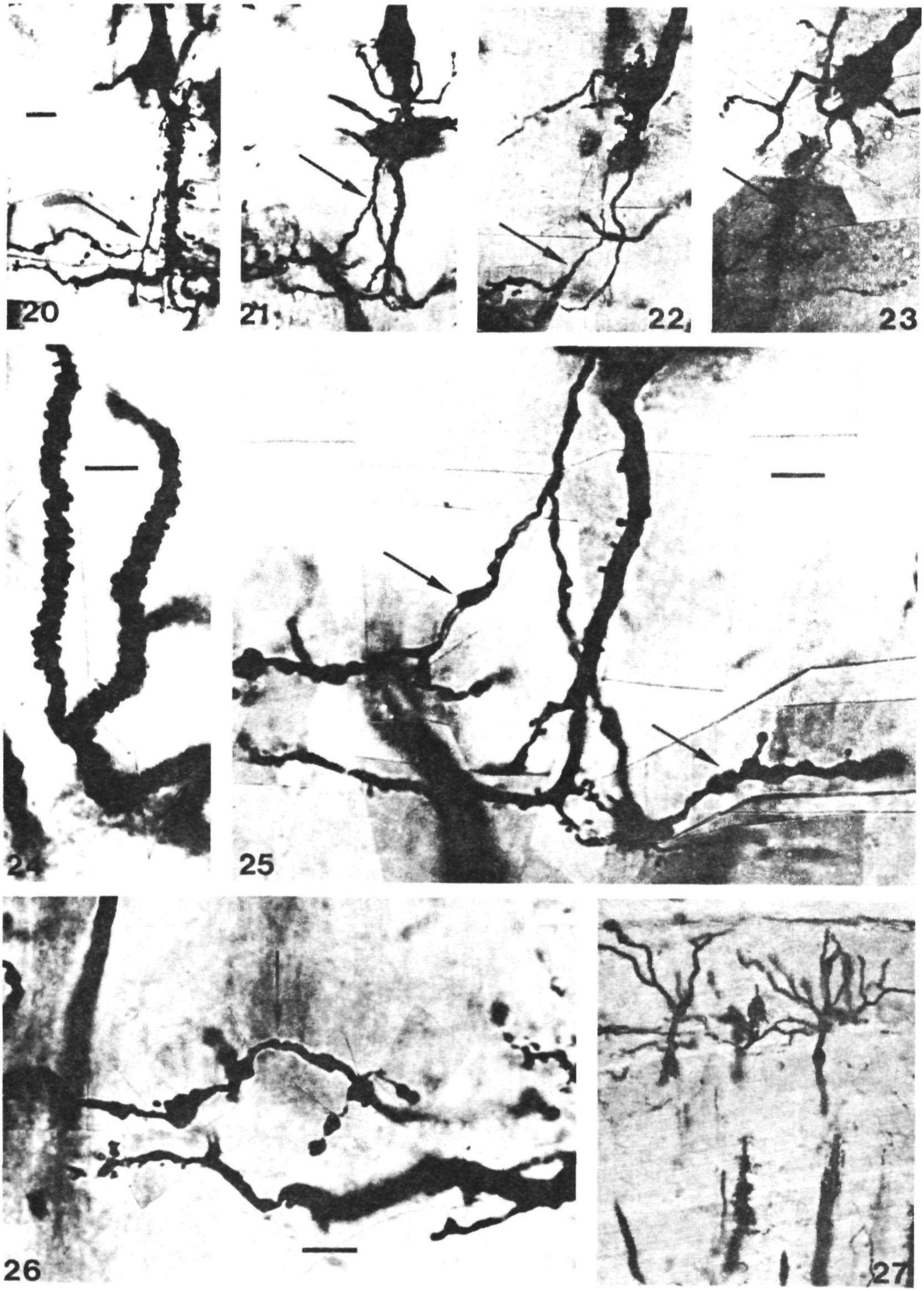
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PLATE 2

EXPLANATION OF FIGURES

28 Type III neuron, Golgi Kopsch Calibration bar 50 μm

29 30 Cell body and axon of two type IVa cells ½ GC + ey The complete cell of figure 30 is drawn in figure 6a Calibration bar 10 μm

31 Type V neuron, ½ GC + ey Calibration bar 50 μm

32 Type VI neuron, thick arrow indicates the unimpregnated continuation of the axon, visible by its diffractive properties Golgi Kopsch Calibration bar 50 μm

33 The initial part of axons of different cell types, impregnated by Golgi Kopsch a, type VI b, type X, c, type XII, d, type I, e, small type XIV, f, large type XIV Notice that the axon of type VI (a) resembles the myelinated axons of type X (b) and XII (c) Unmyelinated axons, e g , of type I (d) and type XIV (e,f) look quite different Calibration bar 5 μm

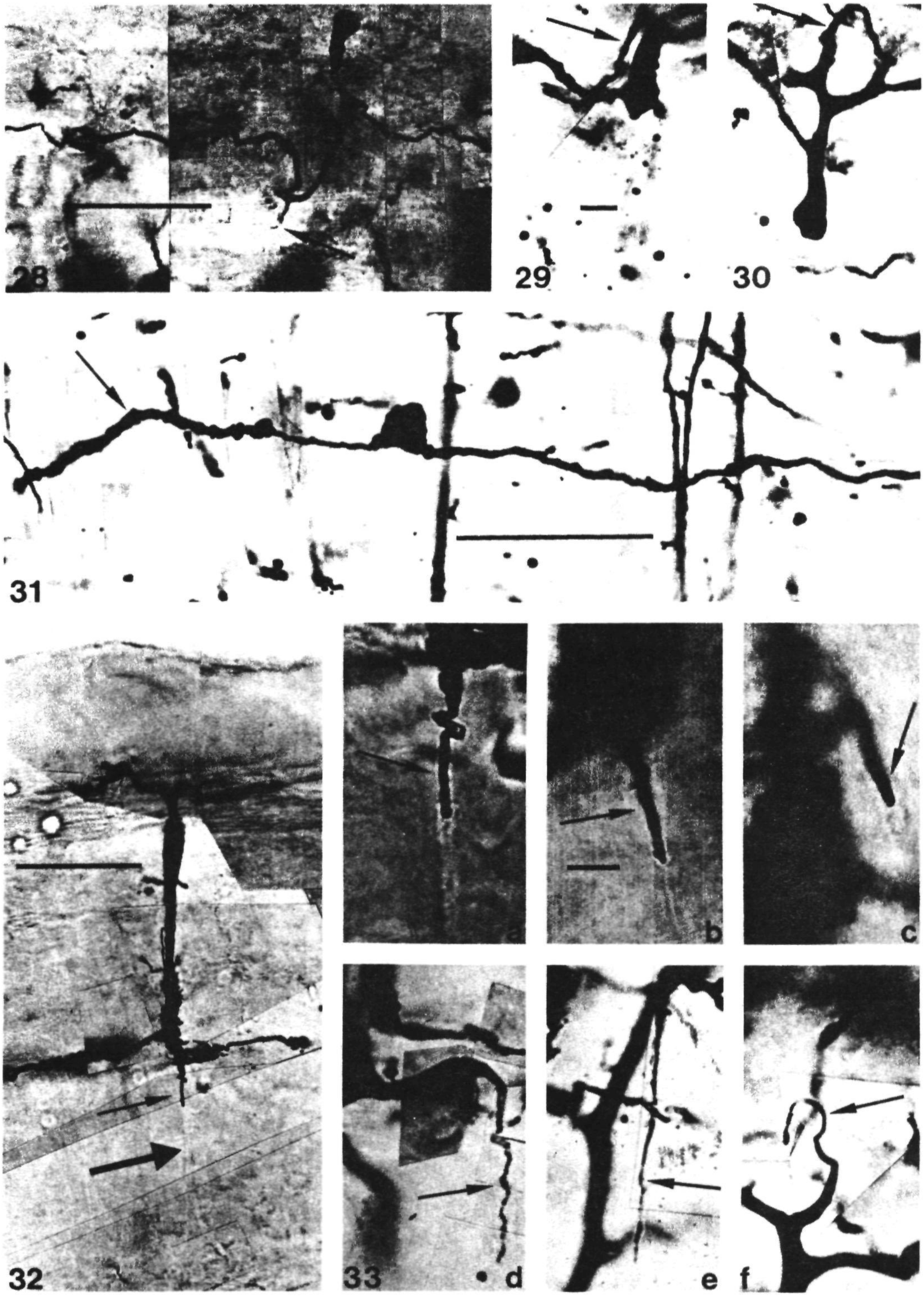
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PLATE 3

EXPLANATION OF FIGURES

34 Type VIII neuron ½ GC + ey Calibration bar 50 μm

35 Axon of type VIII neuron ½ GC + ey Calibration bar see fig 34

36 Cell body, initial axon and impregnated myelin (thick arrow) of a type X neuron ½ GC + ey Calibration bar 10 μm

37 Type $XIII_2$ neuron ½ GC + ey Calibration bar 100 μm

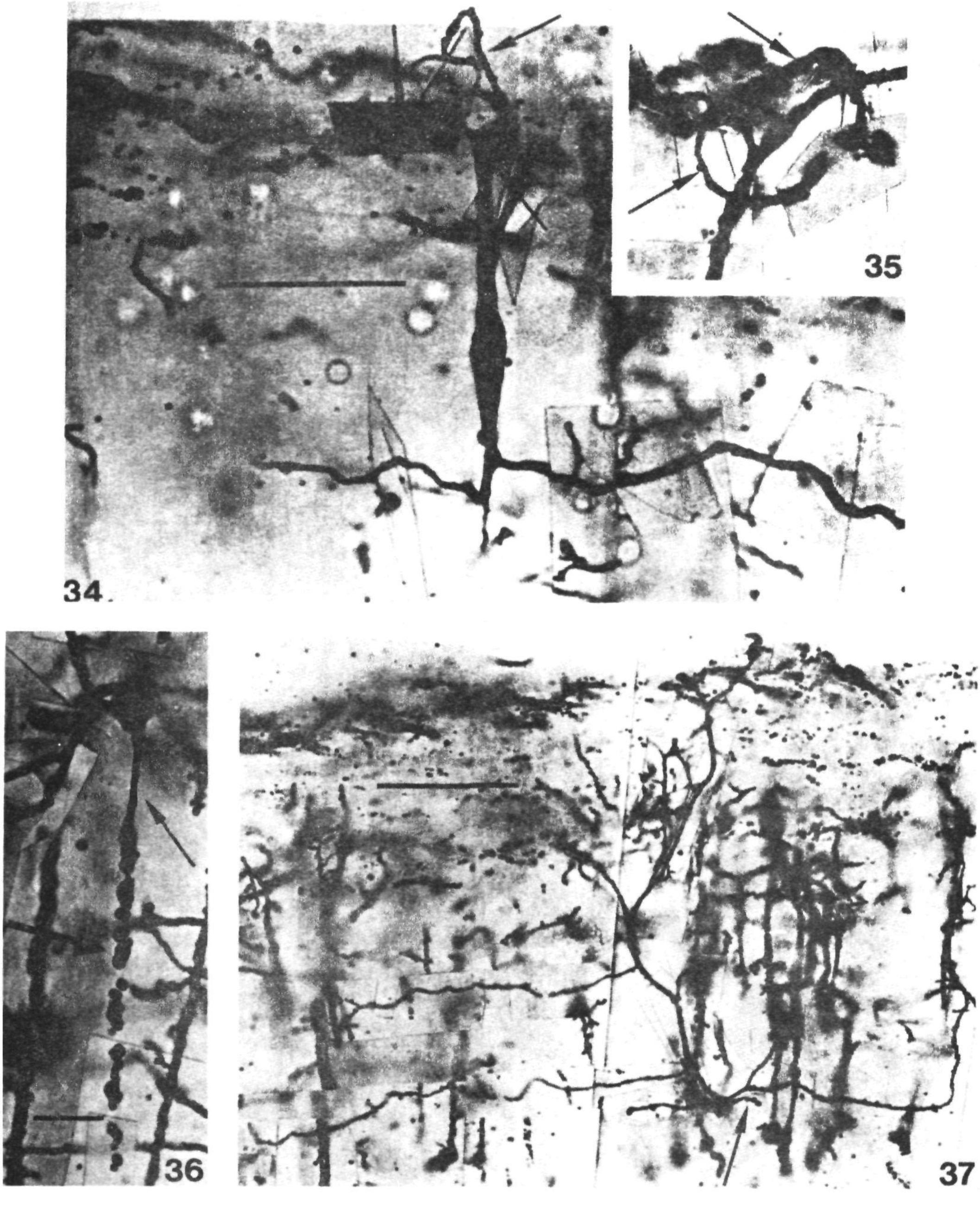
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PLATE 4

EXPLANATION OF FIGURES

38 Type XII cell with an axonal myelin continuation that leaves the tectum (thick arrow) ¼ GC + ey Calibration bar 50 μm

39 Type $XIII_1$ neuron, showing the acriform axonal myelin continuation (thick arrow) ¼ GC + ey Calibration bar 50 μm

40 Axon and apical dendrites of small type XIV cell Notice that the axons lack spines in contrast to the dendrites The "beaded" appearance of the axons resembles that of type I (compare figs 25 and 26), but also the dendrites appear "beaded" Golgi rapid Calibration bar 5 μm


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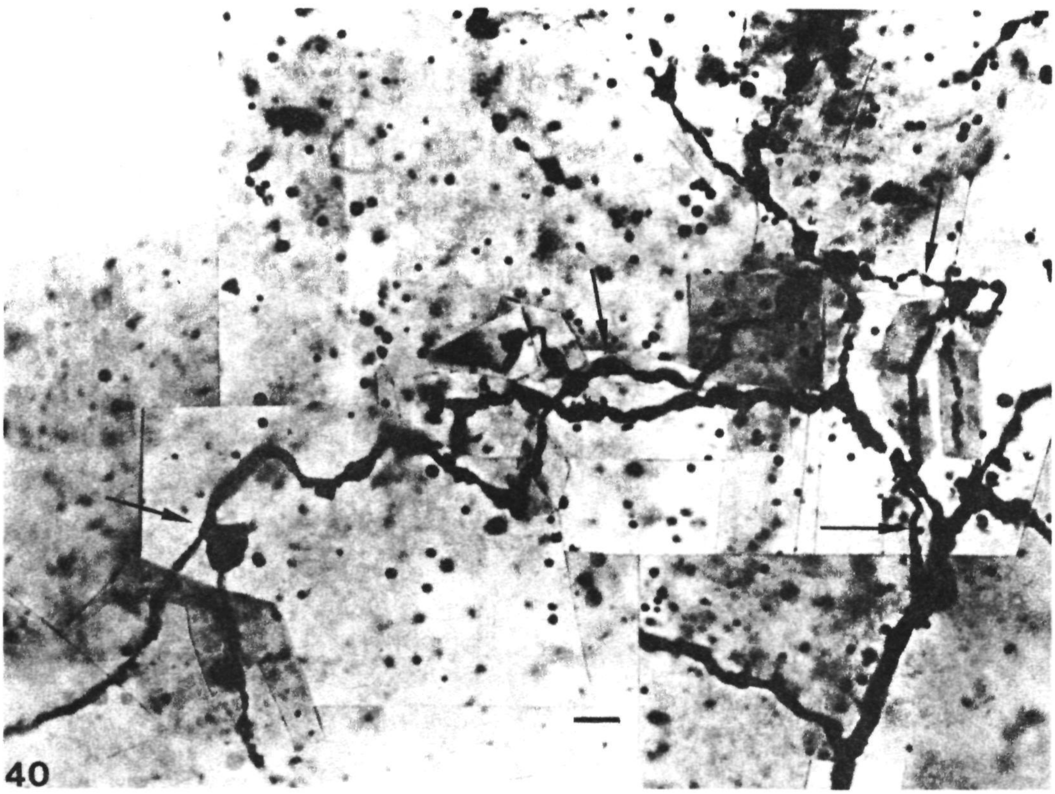
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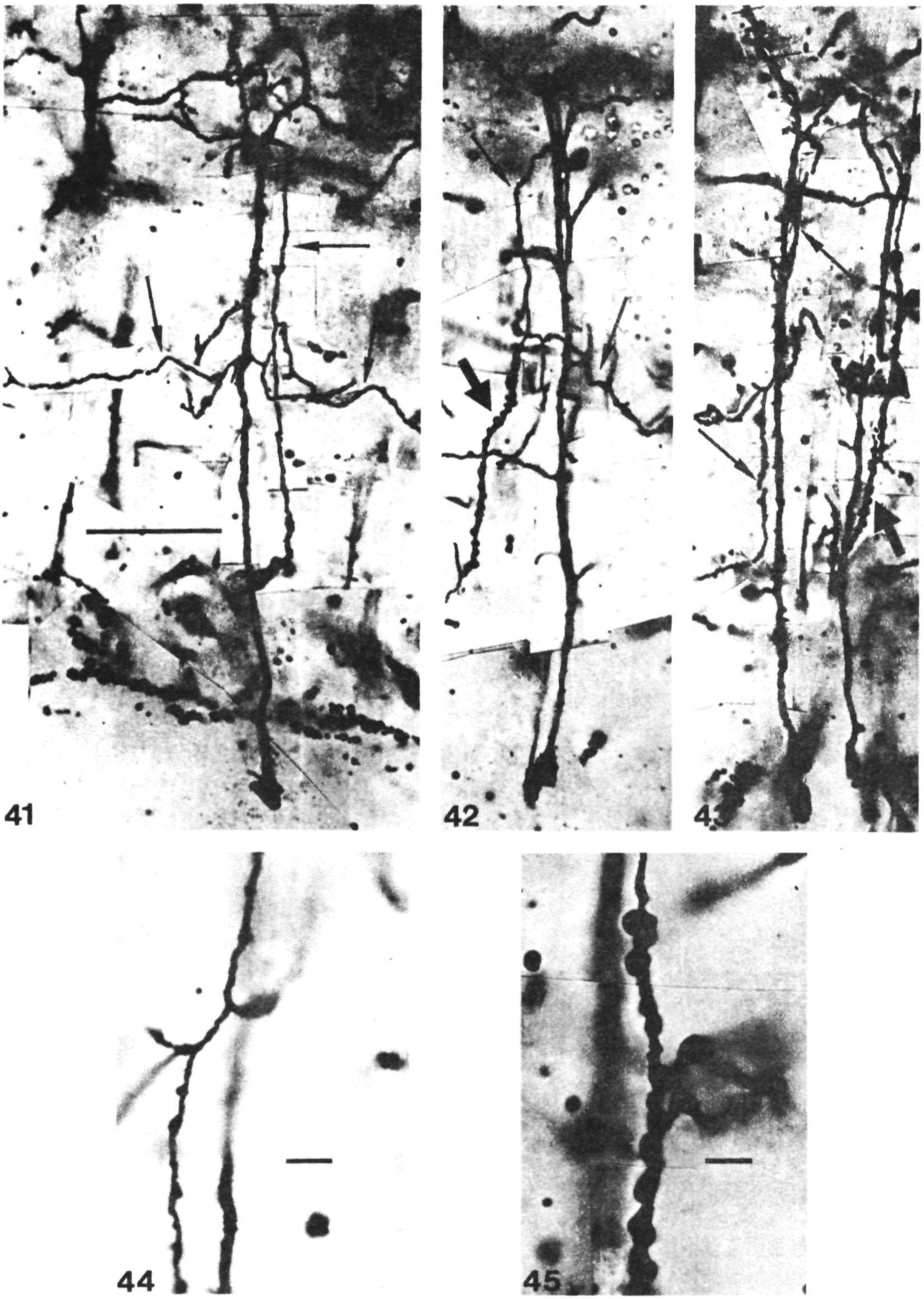
PLATE 5

EXPLANATION OF FIGURES

41-43 Small type XIV cells ½ GC + ey Note that these cells are quite similar, but differ in respect to the axonal "thickenings" (thick arrows) Calibration bar 50 μm

44, 45 Details of the axons in figure 42 Calibration bar 5 μm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CHAPTER IVa

A Golgi-Electron Microscopic Study of Goldfish Optic Tectum. I. Description of Afferents, Cell Types, and Synapses

J MEEK
Department of Morphology, The Netherlands Ophthalmic Research Institute, P.O. Box 6411, 1005 EK Amsterdam, The Netherlands

ABSTRACT A study of goldfish optic tectum was performed with conventional electron microscopy and with the Golgi-EM technique described by Fairén et al. ('77). Five types of tectal afferents, three types of interneurons and three types of efferent neurons were investigated.

Afferents from the torus longitudinalis, which terminate in the marginal layer, contain round synaptic vesicles with a mean diameter of 43 nm. Optic afferents, which terminate in the superficial gray and plexiform layer, are characterized by pale mitochondria with dilated cristae and round vesicles with a mean diameter of 49 nm. Afferents of unknown origin, terminating in several tectal layers, can be subdivided in three types; one containing round vesicles and two containing pleomorphic vesicles with different degrees of ellipticity.

The three types of interneurons studied (type I, III and XIV, of Meek and Schellart, '78) were selected on basis of their high frequency of occurrence. The apical dendrites of type I neurons make many synaptic contacts with the marginal axons. All three types have dendrites in the superficial gray and plexiform layer making contacts with optic nerve terminals. In addition, their dendrites and cell bodies make synaptic contacts with several types of unidentified presynaptic elements. The axon terminals of type I and of type XIV contain round vesicles with a mean diameter of 45 and 46 nm respectively.

Three of the four types of efferent neurons present in the goldfish tectum were studied (type VI, XII and XIII). Two of them make contact with optic terminals (type VI and XII) and two make contact with tectal afferents of unknown origin in the central white layer or in the lower part of the central gray layer (type XII and XIII). The axons of all three types become myelinated at some distance from their origin. Their initial unmyelinated parts are covered with a so-called "outer surface coating", have no collaterals and are occasionally (type VI and XII) or frequently (type $XIII_I$) postsynaptic to other elements. The archiform axons of type $XIII_I$, and to a lesser extent also the shepherds-crook shaped axons of type XII, have a close apposition to looping and narrowing dendrites in the inner plexiform layer.

The present results concerning neuronal circuitry of the goldfish optic tectum are summarized in a tentative scheme.

The midbrain roof of teleosts is a complex brain center with a variety of afferent and efferent connections. The most pronounced afferent system is formed by optic nerve fibers, which mainly terminate retinotopically in the *superficial gray and plexiform layer* (layer 5; Jacobson and Gaze, '64; Schwassman and Kruger, '65; Grafstein, '67; Neale et al., '72; Sharma, '72a; Vanegas and Ebbesson, '73; Landreth et al., '75; Schmidt, '79). A second afferent system comes from the torus longitudinalis, a brain center only present in actinopterygians. The toral fibers contribute to the *marginal layer* (layer 7; Ito and Kishida, '78;

Address reprint requests to J Meek, Department of Anatomy, University of Nijmegen, Postbox 9101, Geert Grooteplein Noord 21, 6500 HB Nijmegen, The Netherlands

Vanegas et al., '79). Furthermore, projections from the telencephalon (Vanegas and Ebbesson, '76; Ito and Kishida, '77; Marotte and Mark, '75), the contralateral tectum (Ebbesson and Vanegas, '76; Grover and Sharma, '79), the nucleus isthmi, the thalamus, the cerebellum and the spinal cord have been described (Ariëns Kappers, '21; Kirsche and Kirsche, '61; Schnitzlein, '64; Marotte and Mark, '75; Ariëns Kappers et al., '67). Four major efferent tracts are described, ascending as well as descending (Ebbesson and Vanegas, '76; Grover and Sharma, '79). The tectal efferents invade pretectal-diencephalic regions, the contralateral rostral tectum, the midbrain tegmentum and presumably also the reticular formation.

Physiological knowledge of the tectum of teleosts is almost exclusively restricted to the visual afferent system. The characteristics of responses to visual stimulation have been studied by many authors (e.g. Jacobson and Gaze, '64; Sutterlin and Prosser, '70; Schellart and Spekreyse, '76; Schellart et al., '79). The optic afferent system has also been used for investigations of axonal transport (see Landreth et al., '75) and neuronal specificity (Sharma, '72b; Levine and Jacobson, '75; Yoon, '75; Cook and Horder, '77; Schmidt, '78; Schmidt et al., '78). Only few reports deal with other sensory modalities, as e.g. auditory tectal responses (Niida, '73) or responses after lateral line stimulation (Guselnikov et al., '64; Callens et al., '67).

Morphological studies on the teleostean tectum using Golgi-impregnation are published by Ramon Cajal (1899), Ramon y Cajal ('11), Leghissa ('55), Vanegas et al. ('74), Schroeder and Vanegas ('77), Meek and Schellart ('78), Romeski and Sharma ('79) and Schroeder et al. ('80). On the basis of dendritic, axonal and somatic characteristics, Meek and Schellart ('78) distinguished fifteen cell types in the tectum of the goldfish (*Carassius auratus*). Up to now three studies have dealt with normal tectal ultrastructure in teleosts (Ito, '70, using *Cyprinus carpio*, Laufer and Vanegas, '74a, using *Eugerres plumieri*, and Ito et al., '80, using *Holocentrus rufus*). They give a detailed description of ultrastructural characteristics and types of synapses in the various tectal layers. The ultrastructural features of optic nerve terminals have been determined with the aid of degeneration techniques in several teleosts, viz. *E. plumieri* (Laufer and Vanegas, '74b); *C. auratus* (Airhart and Kriebel, '80) and *H. rufus* (Ito et al., '80). Additionally, Ito et al. ('80) have characterized degenerating afferents from the telencephalon and contralateral tectum in *H. rufus*.

The present paper first deals with the ultrastructural characteristics of the layers distinguished in the goldfish tectum and with the characteristics of the terminations of tectal afferents. Further, intrinsic tectal cells will be described using a Golgi-EM procedure, with special emphasis on pre- and postsynaptic elements. For this purpose three types of interneurons and three types of efferent neurons are selected. The interneurons represent the most frequently occuring types, while the efferent neurons represent three of the four types present (Meek and Schellart, '78).

In a subsequent paper (Meek, '81) I will deal with quantitative aspects of the synaptology of the cell types described in the present paper.

METHODS

Histological procedure

Common goldfish (*Carassius auratus*), 18-25 cm long, were used. After exposure of the tectum of anaesthetized fish (MS 222, 0.33 g/l, 10 minutes), the animal was sacrificed by cutting the medulla oblongata. Within the next minute the tectum was removed, together with other mesencephalic structures, and immersed in a fixative for 3 hours at 20°C. Two fixatives were used; 2% glutaraldehyde/2% paraformaldehyde in 0.07M phosphate buffer after Sörenson or 5% glutaraldehyde / 5% paraformaldehyde in the same buffer. Following prefixation, the impregnation/de-impregnation method of Fairén et al. ('77) was applied, except for sectioning, which was done according to Blackstad ('75). In short the procedure was as follows: (a) *Golgi-impregnation;* immediately after prefixation the tecta were immersed for two days in a mixture of 0.2% osmium tetroxide and 2% potassium dichromate in distilled water (20°C) followed by two days impregnation in 0.75% silver nitrate (20°C) (b) *Sectioning;* 50 μm serial sections were cut on a *Vibratome*, the trough being filled with 50% ethanol saturated with silver chromate (5-10°C). The sections obtained were quickly studied in the light microscope (LM) and selected for the de-impregnation procedure on the basis of the presence of completely impregnated cells of the different types. The remaining sections were made permanent via ethanol, xylene and malinol. The correct sequence was carefully maintained. (c) *Gold-toning;* the selected sections were quickly washed in distilled water, immersed for 15 minutes in 0.05% hydrogen tetrachloroaurate (HCl_4 Au H_2O) at 5-10°C, washed for 5-10 minutes in distilled water at 5-10°C (three changes), immersed for three minutes in 0.05% oxalic acid at 5-10°C and washed for 15 minutes in distilled water (three changes). Finally the sections were warmed up to 20°C in distilled water. (d) *De-impregnation;* the sections were immersed for 30-45 minutes in 1% sodium thiosulfate at 20°C (two changes) and washed for five minutes in distilled water (two changes). After this they were transferred to 0.1 M cacodylate buffer, in which they were stored for a few hours up to one day. (e) *Postfixation;* immersion for 30 minutes in 2% osmium tetroxide in 0.1 M Veronal buffer at 20°C. (f) *Dehydration and embedding;* the sections were quickly (in 75 minutes) dehydrated in a graded series of ethanol and, via propylene oxide, embedded in Epon 812. Embedding was carried out on slides, and the sections were covered with cover slips.

Light microscopy

The de-impregnated sections, embedded in Epon 812 between a slide and a cover slip, were studied in a light microscope, equipped with a drawing apparatus. When necessary, adjacent non-de-impregnated sections could be studied. To facilitate trimming for electron-microscopic (EM) sectioning, an overall picture of the 50 μm section was drawn at a magnification of about × 25, and structures of interest were indicated. Cells selected for reconstruction in the EM were drawn at a magnification of about × 400. The depth of the different components in the section was indicated in the drawing using the micro-screw reading. Besides, a drawing was made of de-impregnated structures in the neighbourhood of the cell of interest. In a few instances complex details were drawn at a magnification of about × 1000. At this stage photographs were taken as well. At least a complete through-focus series of each cell at a magnification of about × 400 was made. It should be noted that the use of Vibratome slices and the subsequent investigation of tissue also allows selection of non-impregnated structures. This was the case for several archiform axons of type $XIII_1$ neurons, which were outstanding by their osmicated myelin sheaths.

Electron microscopy

To allow thin sectioning, the cover slip was carefully removed from the Epon by means of a sharp scalpel, which was put between the Epon and cover slip. After some experience, the cover glass indeed could be removed in small pieces, leaving the embedded section undamaged. Next, the Epon sheath with the 50 μm section was removed from the object glass by a scalpel and divided into several pieces. Pieces of interest were mounted upon prepolymerized Epon blocks with the aid of liquid Epon, which then was allowed to polymerize.

The final block was trimmed to a pyramid under oblique illumination from below, which visualizes de-impregnated structures satisfactory. After trimming, serial sections with white interference color (60-90 nm) were cut and mounted on 75-mesh copper grids, covered with a formvar/carbon film. On each grid ten serial sections were mounted. After 70 or 80 sections the pyramid was viewed under the light-microscope using oblique illumination. In this way it could be ascertained which cellular components were cut in the preceding sections. The advantage of this procedure, compared with intervening sectioning of 1 μm sections, is that no material is lost for EM analysis.

Thin sections were contrasted with uranyl acetate and lead citrate and studied in a Philips EM 400. At first, cells of interest were reconstructed using photomontages at low magnification (about × 2000) obtained from one section of each grid. For this purpose the light-microscopic drawings and photographs, and the drawings made during thin sectioning were of great help. If necessary, e.g. to trace small dendrites or axons, reconstructions were made at higher magnification (about × 16,000), using three or more sections on each grid. Apart from the de-impregnated neurons, some unimpregnated neurons present in the sections studied could be reconstructed and identified. In a second stage, the identified and localized structures were studied at high magnification to investigate synapses and cytoplasmic characteristics.

For the investigation of synaptic vesicles, the lengths of their largest axis and of the axis perpendicular to this were measured on photographs with a final magnification of about × 30,000. An accurate measuring loupe was used for this and the exact magnification was determined using a grating replica. The mean of both axes (per vesicle) was taken as a measure for the size, and the ratio between the long and the short axis was taken as a measure for the ellipticity. The different prefixations used had no significant influence on size and shape of the vesicles. The significance of differences in size and shape of the vesicles was tested by means of the chi-square statistical test.

General characteristics of de-impregnated tectal structures

The precipitate which results from Golgi-impregnation and subsequent de-impregnation, has in our tissue the same appearance as originally described by Fairén et al. ('77) and consists of small electron-dense grains scattered in the cytoplasm. In cell bodies and large dendrites the precipitate is almost exclusively restricted to a region near the cellular surface, while in small dendrites and axons the precipitate is present throughout the profile. Grains are absent in nuclei and mitochondria.

In material fixed in 5% glutaraldehyde / 5% paraformaldehyde the precipitate is precisely restricted to the impregnated structures, but in tecta fixed with a solution containing 2% of both aldehydes, a rim of precipitate is often present around impregnated cell bodies or large dendrites (cf. Figs. 18 and 19).

The impregnation / de-impregnation procedure appears to leave most organelles unaltered. For instance, impregnated and unimpregnated optic terminals have mitochondria with a similar ultrastructural appearance (cf. Figs. 15 and 7) and their vesicles have the same size and shape (tested with chi-square). Other cytoplasmic contents, like rER and Golgi cisternae, equally have no deviating appearance in de-impregnated structures (e.g. Figs. 20 and 21). However, postsynaptic membranous densities are less outstanding when the postsynaptic element is impregnated. This implies that a distinction between symmetric and asymmetric contacts is very precarious.

For the de-impregnated cells selected for this study, no completely un-impregnated dendrites were observed. However, small parts of somatic or dendritic cytoplasm were sometimes devoid of precipitate. The boundary between the impregnated and unimpregnated part of the cytoplasm is rather sharp in such cases (Fig. 13). In contrast, axons are frequently incompletely impregnated and for myelinated axons the impregnation is absent as soon as the myelin sheath starts (Figs. 38 and 39). Incidentally, a single presynaptic terminal that makes contact with an impregnated soma or large dendrites is also impregnated (Fig. 14). In such terminals the precipitate has a different appearance. It does not consist of fine grains but of larger, cristalline structures.

RESULTS

General layer composition

The tectal layers, numbered from inside outwards 1 to 7 (Fig. 1) are easily recognized in the EM (Figs. 2 and 3).

Layer 7, *the marginal layer,* consists predominantly of unmyelinated axons. These run parallel to the tectal surface and frequently make asymmetric synapses with spines of dendrites that traverse this layer obliquely (Figs. 2 and 4).

The *optic layer,* layer 6, is composed of bundles of myelinated fibers separated by dendritic trunks that run perpendicular to the tectal surface (Fig. 2). The fibers originate for the major part in the retina (e.g. Sharma, '72a; Neale et al., '72; Laufer and Vanegas, '74b and Landreth et al., '75). Their diameters range from 0.6 to 6 μm and their mitochondria have characteristic dilated cristae and a rather electron-lucent matrix (Fig. 5).

Layer 5, *the superficial gray and plexiform layer,* is constituted by a complicated neuropil, interspersed with neuronal cell bodies and myelinated axons (Fig. 2). Layer 4, *the inner plexiform layer,* has at low magnification about the same appearance as layer 5, with the exception that myelinated fibers are only incident-

Abbreviations

G	Golgi-complex
isc	inner surface coating (=subsurface coating)
m	mitochondrion
mf	microfilaments
mt	microtubules
my	myelin
N	nucleus
nu	nucleolus
osc	outer surface coating
rER	rough endoplasmic reticulum
sv	synaptic vesicles

Fig. 1. Semithin section of goldfish tectum, showing the tectal layers indicated after Meek and Schellart ('78). Calibration bar: 100μm.

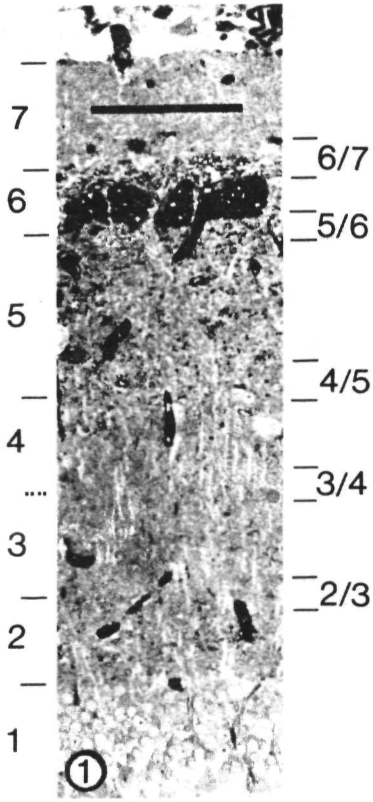
Fig. 2. Low power electron micrograph with parts of layer 5, 6 and 7. Calibration bar: 10μm.

Fig. 3. Low power electron micrograph with parts of layer 1, 2 and 3. Calibration bar: 10μm.

Fig. 4. Detail of layer 7, showing a dendritic profile and spines, which make synaptic contacts with marginal axon terminals. Calibration bar: 0.5μm.

Fig. 5. Detail of layer 6, showing several myelinated optic nerve fibers with their characteristic mitochondria with dilated cristae and a rather electron-lucent matrix. The disrupted aspect of the myelin is presumably due to the fact that immersion fixation was used. Calibration bar: 0.5μm.

Fig. 6. Detail of layer 1, showing close apposition of two periventricular neurons with gap-junction-like membranous specializations (arrows). Calibration bar: 0.5μm.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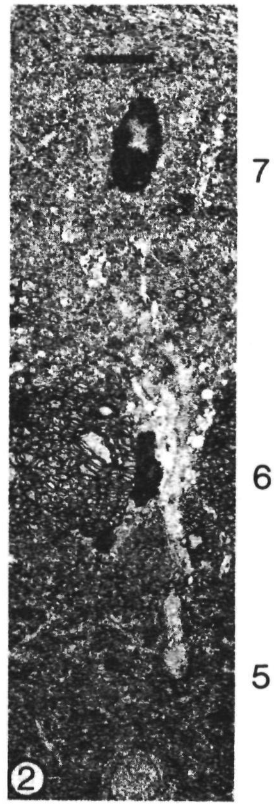
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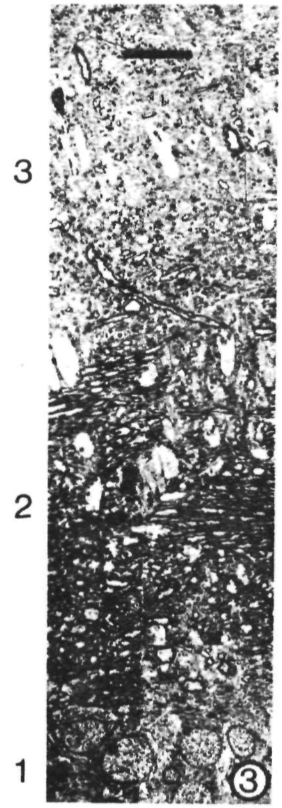
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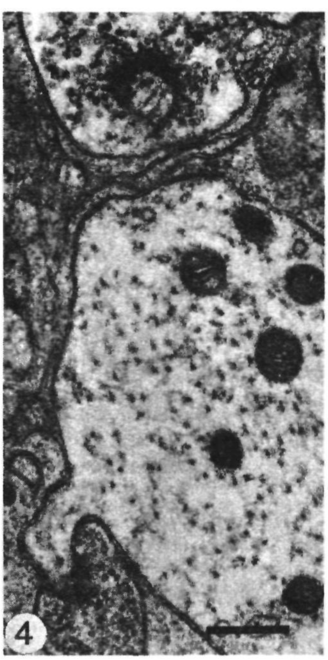
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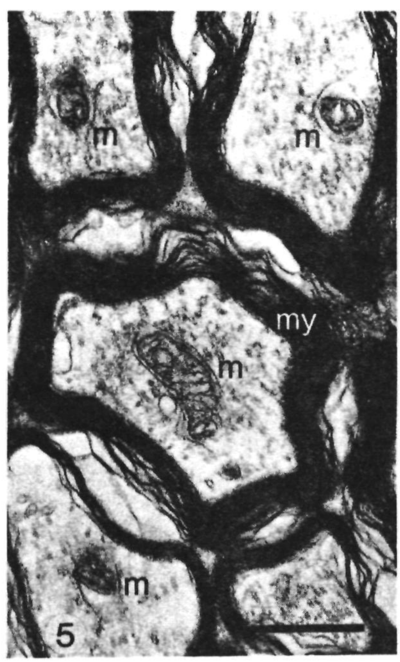
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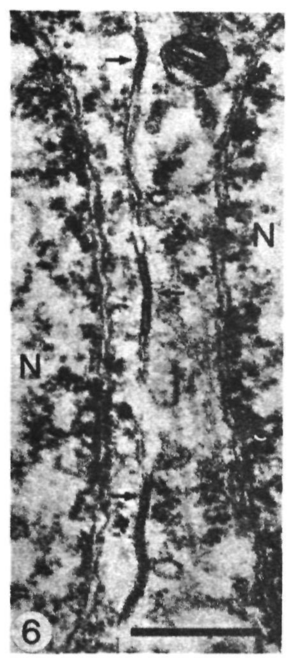
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ally observed. In the boundary region between layer 5 and 4, bundles of myelinated fibers with relatively large diameters are present (Fig. 9). Because of the similarity in fine structure, the boundary between layer 4 and 3, which together form the *central gray layer,* is very diffuse. The number of myelinated fibers gradually increase in deeper parts of layer 3.

Layer 2, *the central white layer,* partly consists of myelinated nerve bundles. These are less densely packed than in layer 6. The remaining part of this layer consists of neuropil (Fig. 3).

Layer 1, *the periventricular gray layer,* is composed of small, densely packed neurons (Figs. 1 and 3). At several places, bundles of myelinated and unmyelinated fibers traverse this layer obliquely, and the apical processes of the deeper cells separate clusters of the more superficial cells. The cellular membranes of the periventricular cell bodies are closely apposed and show membranous specializations resembling gap junctions (Fig. 6). The most superficial somata are incidentally contacted by presynaptic elements, whereas deeper cell bodies are devoid of such contacts.

Characterization of some tectal afferents

Before describing the de-impregnated material, some introductory observations on unimpregnated tectal afferents will be presented. Myelinated axons that leave their myelin sheaths and make presynaptic terminals are considered as tectal afferents, since interneurons with myelinated axons are absent and efferent neurons have no collaterals in the tectum (Meek and Schellart, '78). In layers 2-6, unmyelinated afferents cannot be distinguished from axons of interneurons. However, in layer 7, axons of interneurons do not occur (Meek and Schellart, '78). Consequently, all terminals in this layer arise from the unmyelinated marginal axons, which appear to originate in the torus longitudinalis (Ito and Kishida, '78). In our sample all terminals investigated in layer 7 are of one, specific type, called type A in Figure 16 (see also Figs. 4 and 23).

Optic nerve fibers predominantly terminate in layer 5 (Sharma, '72a; Grafstein, '67; Neale et al., '72 and Landreth et al., '75). Their terminals are characterized by the presence of pale mitochondria with dilated cristae, which were already described for the fibers in layer 6. For several optic terminals, the axonal origin could be ascertained by their sprouting from a myelinated axon (Figs. 7 and 8). These terminals contain large round vesicles and are designated as type B in figure 16. Some terminals are *"en passant"* (Fig. 9). The vesicles in optic nerve terminals are significantly larger than the vesicles in terminals of toral fibers, and the mean vesicle size per terminal does not overlap for these two types of tectal afferents (Fig. 16). The large round vesicles, together with the pale mitochondria with dilated cristae allow the identification of optic nerve terminals even when their myelinated origin is absent in the sections studied (see e.g. Figs. 15 and 36). Optic nerve terminals make asymmetric synapses, predominantly with dendritic profiles. However, small profiles containing synaptic vesicles themselves can be postsynaptic to optic terminals as well. The vesicles in these profiles are round and have an average diameter of 43 nm (Fig. 8).

In layer 5, several other types of myelinated axon terminals are observed (type C, D and E in Fig. 16). Terminals designated as type C contain the same kind of vesicles as the optic terminals, but their mitochondria have either an electron dense matrix and normal cristae, or are densely filled with narrow cristae leaving little space for a matrix (Fig. 10). Type D and E terminals, containing pleomorphic vesicles, are only occasionally present. During random scans through layer 5 and 6, to select terminals arising from myelinated axons, fifteen optic nerve terminals (type B), five terminals of type C and one terminal of type D and E each were

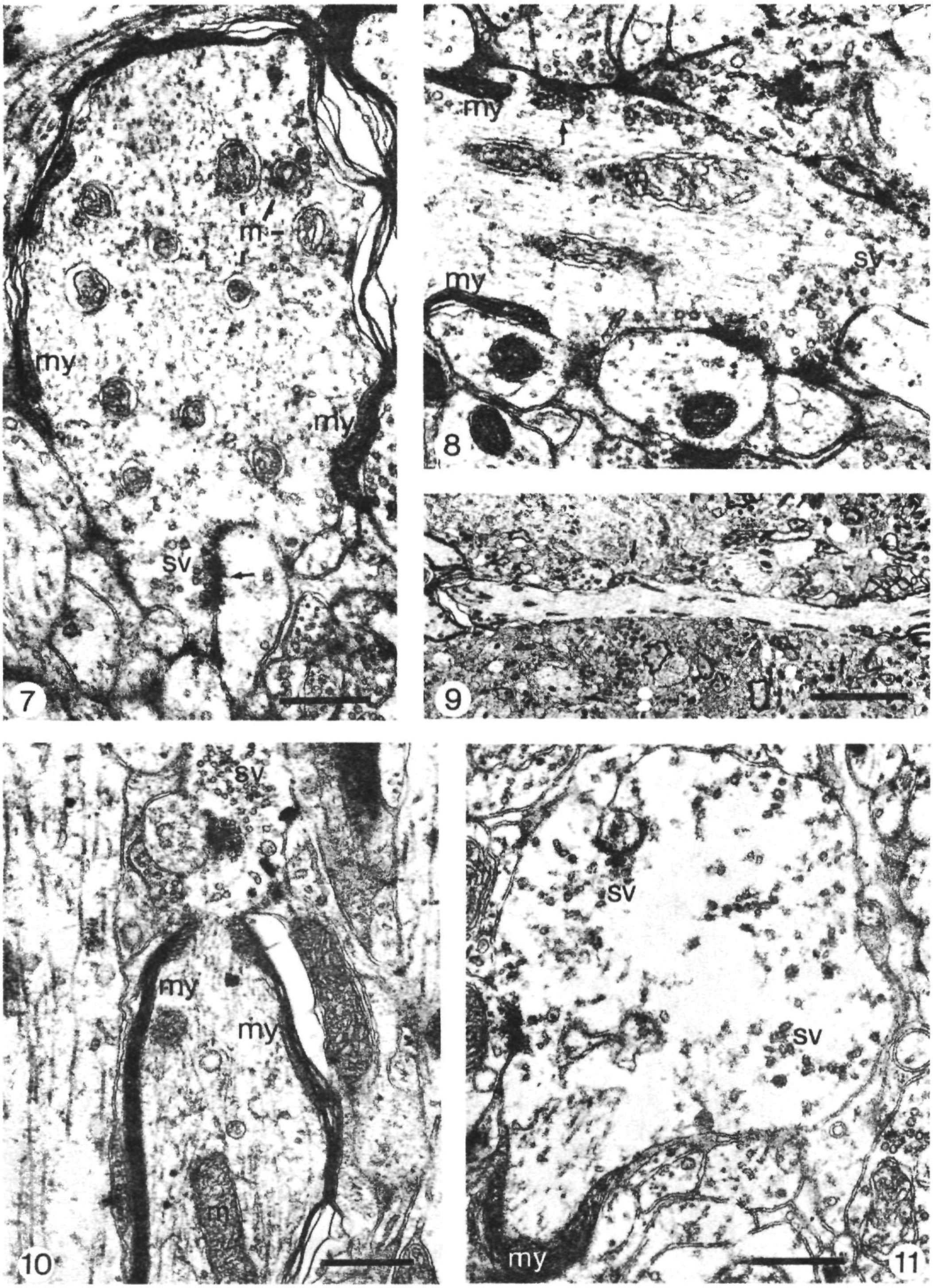
Fig. 7. Optic nerve fiber with its characteristic mitochondria, leaving its myelin sheath to make a synaptic contact (arrow). Calibration bar: 0.5 μm.

Fig. 8. Optic nerve fiber, leaving its myelin sheath to make several synaptic contacts, among which one (arrow) with a profile clearly containing synaptic vesicles. Calibration bar: see Figure 7.

Fig. 9. Large optic nerve fibers in layer 4/5 at low magnification. One of them is unmyelinated for some length and makes synapses *en passant* at the sites indicated by arrows. Calibration bar: 5 μm.

Fig. 10. Axon terminal of type C (for characteristics, see Figure 16), located in layer 5. The synaptic vesicles resemble those of optic terminals, but the mitochondria are different. Calibration bar: 0.5 μm.

Fig. 11. Axon terminal of type E (for characteristics, see Figure 16), located in layer 3. The terminal contains pleomorphic vesicles. Calibration bar: 0.5 μm.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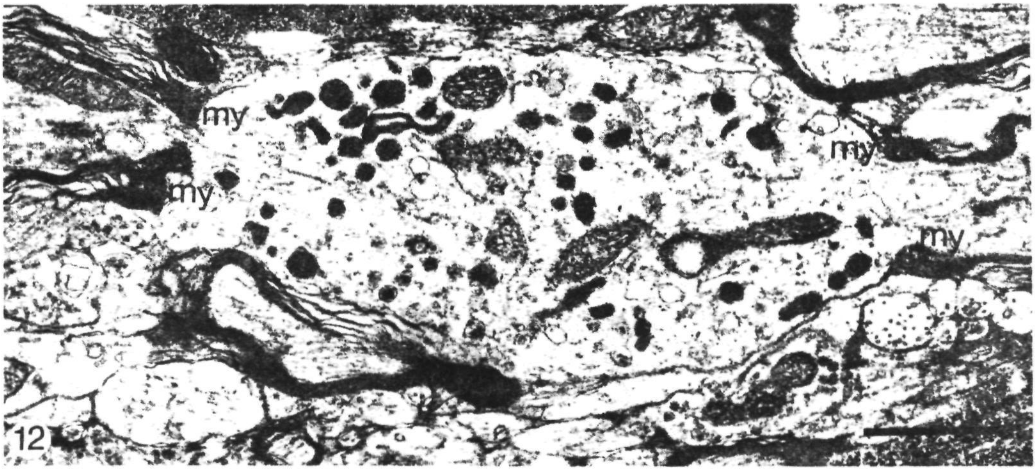
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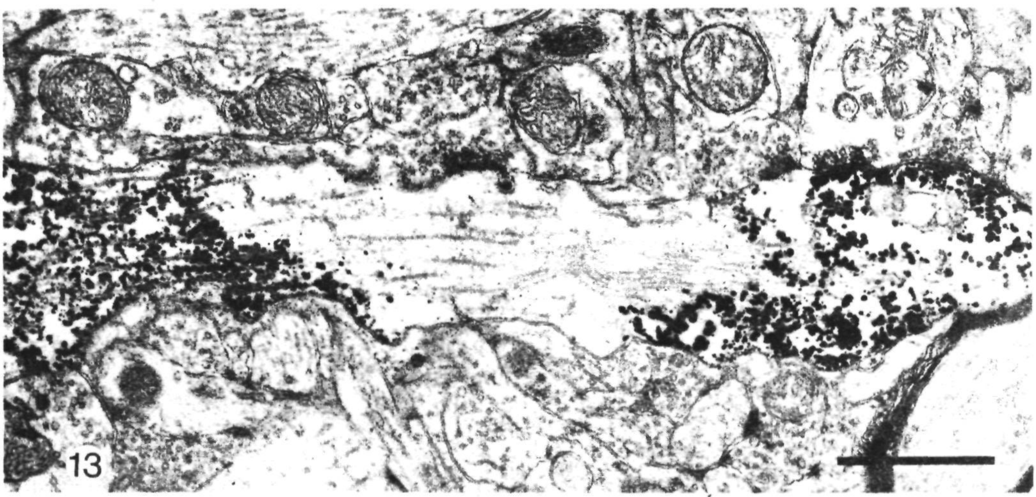
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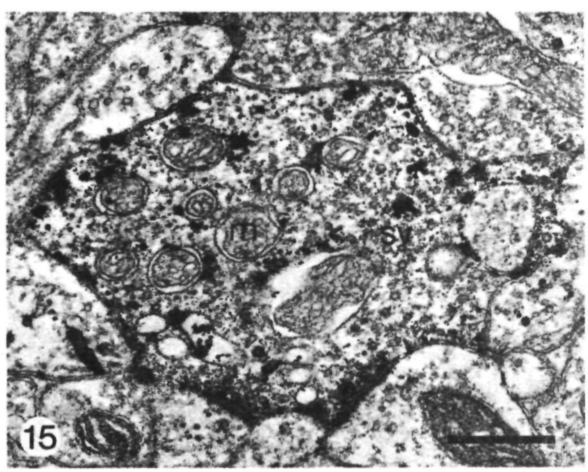
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Fig 12 A myelinated axon in layer 2, making a *bouton en passant* containing large granules with an electron dense content Calibration bar $1 \mu m$

Fig 13 Example of incomplete impregnation, as observed in a dendrite of a type XIII, cell Calibration bar $1 \mu m$

Fig 14 Example of "presynaptic element impregnation" (see Methods) Notice that the precipitate in the terminal is rather cristalline, in contrast to normal de-impregnated tissue (cf e g the postsynaptic element or the bouton of Figure 15) Calibration bar $0.5 \mu m$

Fig 15 De-impregnated optic nerve terminal, showing the same type of mitochondria and synaptic vesicles as unimpregnated examples (cf Fig 7 and 8) Calibration bar $0.5 \mu m$

observed. These numbers roughly indicate their ratio of occurrence. All of these terminals were found in layer 5 except one of type C in layer 6.

In layers 2 and 3, terminals of type C, D and E were observed (Figs. 11, 45 and 46). The distinction between type D and E is based on the distribution of the mean ellipticity index per terminal (Fig. 16), which shows more or less three clusters: one for round vesicles (values varying from 1.08 to 1.20), and two for pleomorphic vesicles (values varying between 1.29 and 1.39, and 1.45 and 1.54 respectively). Shape as well as size of the vesicles of type D and E are significantly different (tested with chi-square). During random scans in layer 2 and 3, seven terminals of type C, six of type D

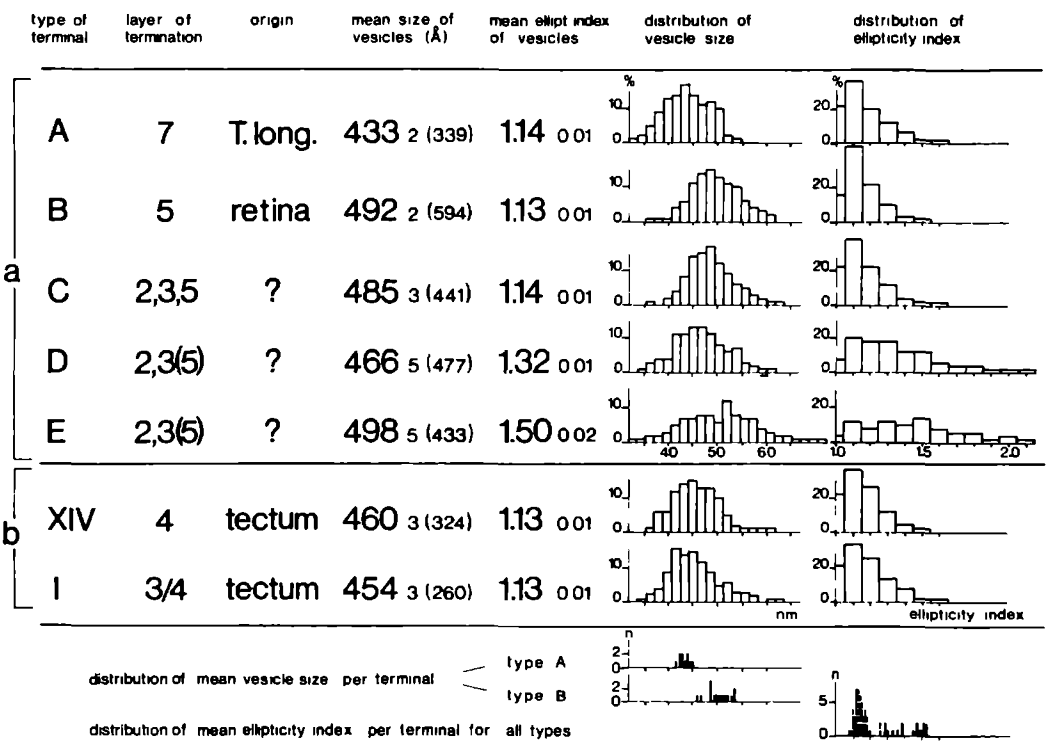


Fig 16 a) Characteristics of the terminals of some tectal afferents The fourth and fifth column indicate the mean value with the standard error of the mean In parentheses, the number of vesicles studied is given b) Characteristics of the axon terminals of two types of tectal interneurons

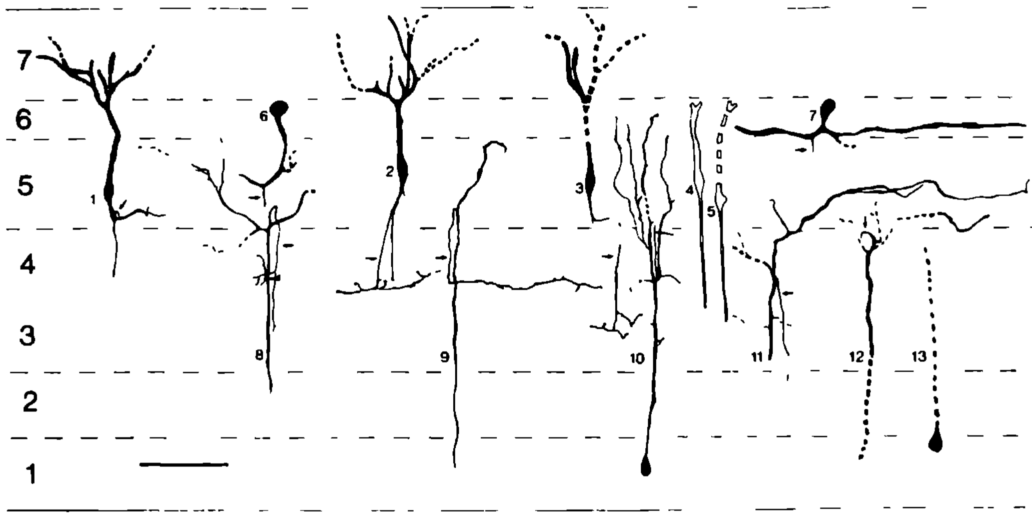


Fig 17 Composite drawing of the interneurons studied in this paper *Black* indicates those parts of de-impregnated neurons that were studied in the EM, *Dotted* parts represent de-impregnated cellular elements which were not studied in the EM and *White* parts represent unimpregnated structures that were studied in the EM The numbers indicate 1-5, type I cells, 6 and 7, type III cells and 8-13, type XIV cells The classification is according to Meek and Schellart ('78) The arrows indicate axons The calibration bar 100μm

and also six of type E were encountered. Apart from this, in layer 2 one axon was observed that formed a *bouton* containing large granules with an electron dense content (Fig. 12). Isolated profiles with such large dense granules are occasionally present in the layers 2 to 5. Optic nerve terminals were not observed in layer 2 and 3.

Characteristics of three types of interneurons

Golgi pictures of the cells to be described are given in Figure 17. Their description is not exclusively based on de-impregnated cells, but also on some un-impregnated cells that could be identified and reconstructed in the EM. The cell types are indicated with Roman numerals according to Meek and Schellart ('78), to which the reader is referred for a detailed characterization of the cell types.

Type I neurons have a bipolar cell body in layer 5. They have a pronounced dendritic tree in layer 7 and dendrites in the lower part of layer 5 and in layer 3/4. The axon originates in layer 4/5 and terminates in layer 3/4.

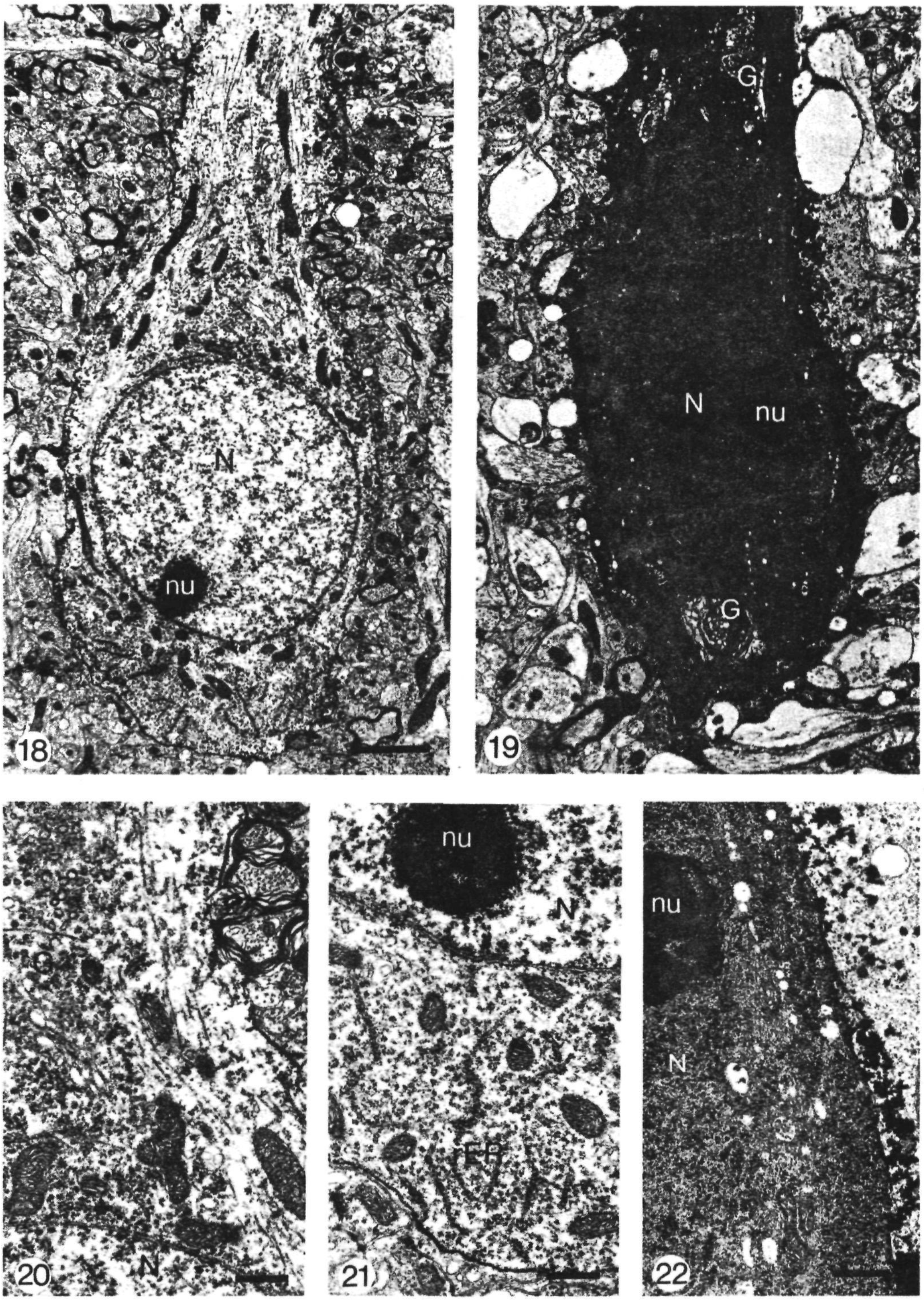
The ultrastructural appearance of the cell bodies depends on the prefixation used. When fixed with a solution of 5% glutaraldehyde / 5% paraformaldehyde they have a clear cytoplasmatic matrix in which organelles are easily recognized (Fig. 18). After fixation in 2% glutaraldehyde / 2% paraformaldehyde, type I cells have a dark appearance. In extreme cases the membranes of the nucleus, cytoplasm and mitochondria are hardly visible, and Nissl substance is not visible at all (Figs. 19 and 22). Dark cytoplasm is also present in the initial dendrites and axon, but more distally located structures are not dark (e g. axon collaterals in layer 3/4 or dendrites in layer 7). It should be noted that this phenomenon is observed for impregnated as well as unimpregnated type I cells, but is absent in all other types of neurons studied, even in type VI, the cell body of which has approximately the same shape and location as type I.

Fig 18 Cell body of a de-impregnated type I cell (Fig 17, cell 1), prefixed in 5% paraformaldehyde/5% glutaraldehyde Calibration bar 2μm

Fig 19 Cell body of a de-impregnated type I cell (Fig 17, cell 2), prefixed in 2% paraformaldehyde/2% glutaraldehyde Calibration bar see Figure 18.

Figs 20 and 21 Details of a neighbouring serial section of Figure 18, showing a part of the apical cytoplasm with a Golgi system (Fig 20) and a part of the basal cytoplasm with granular endoplasmatic reticulum (Fig 21) Calibration bar 0 5μm

Fig 22 Detail of a neighbouring serial section of Figure 19, showing the darkened cytoplasm and impregnation artefacts at the outside of the cellular membrane Calibration bar 0 5μm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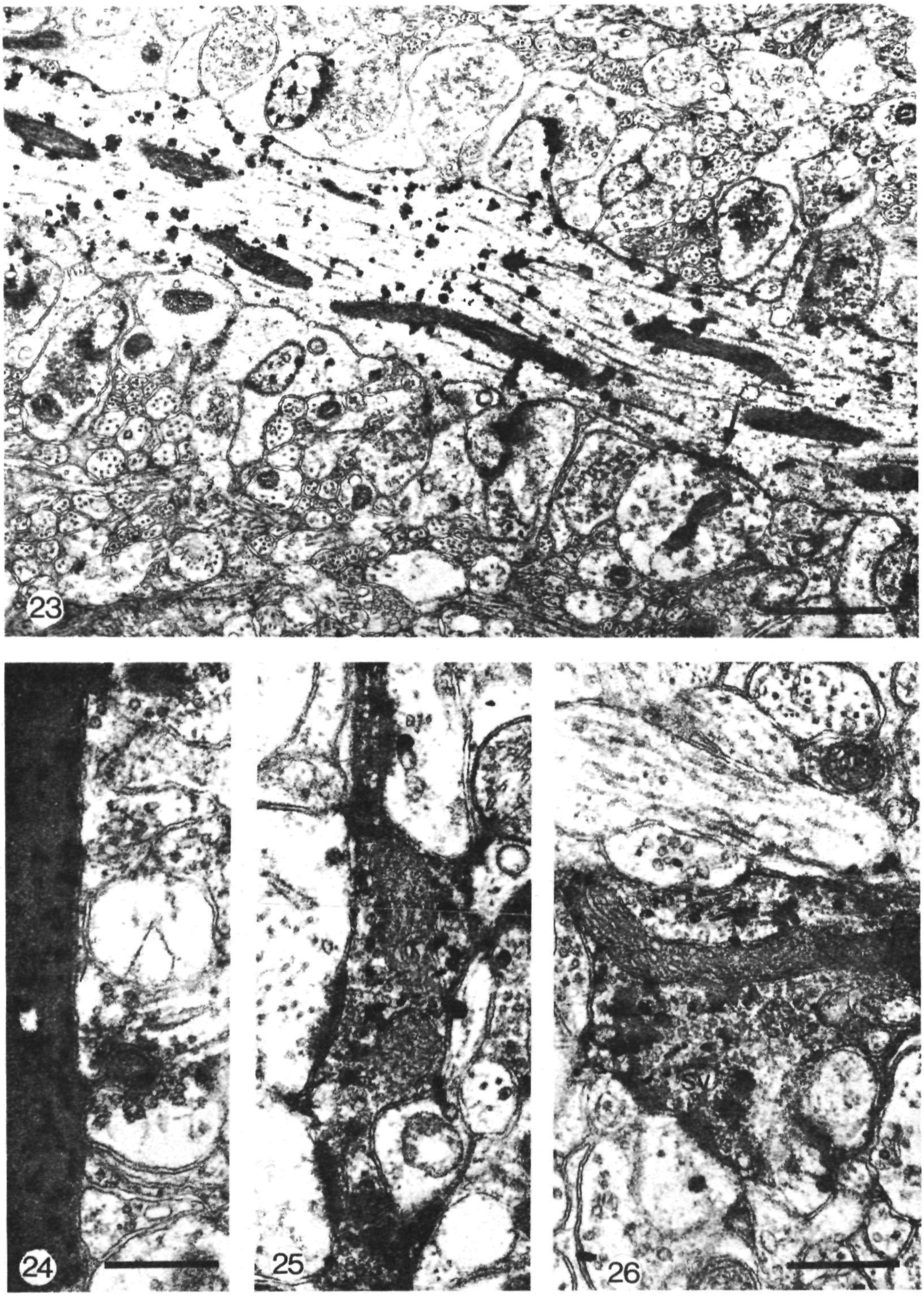
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The somatic cytoplasm of type I neurons contains rough endoplasmic reticulum (rER), polysomes, microtubules and mitochondria with rather electron-dense matrices (Figs. 20 and 21). Such mitochondria are present in all parts of type I cells, (Figs. 23, 25 and 26). In the soma and proximal parts of the dendritic shafts Golgi-complexes occur (Fig. 20). The round nucleus contains one nucleolus (Figs. 18 and 19). The initial parts of the dendritic shafts contain the same organelles as the cell body, but in distal parts microtubules become the most pronounced constituents. Synapses on the cell body and dendritic shafts are rare.

The dendrites in layer 7 have many spines, which make contacts with the marginal axons (Fig. 23) The marginal axons also terminate incidentally between the spines (Fig. 23) The dendrites in layer 4 and 3/4 have some spines as well (Fig. 24), but their spiny aspect is less pronounced than that of the apical dendrites, and most synapses are found directly upon the dendritic shaft. Upon the dendrite in layer 5 studied, some optic nerve terminals have been observed

The descending part as well as the horizontally running collaterals of the axon of type I neurons form terminals in layer 3/4 (Figs 25 and 26). Most synaptic contacts occur on "beads", but they are also present in between these swellings (Figs. 25 and 26). The terminals contain medium sized, round vesicles (Fig. 16b)

Type III neurons have a cell body in layer 6 and horizontally running dendrites in layer 6 or in the superficial part of layer 5. The axon arises from one of the dendrites and initially runs towards deeper tectal layers Axon terminals could not be studied in the EM since they were not impregnated with the method used.

The cell body contains a round nucleus with one nucleolus and all organelles normally present in neurons (Fig. 27). The endoplasmic reticulum is somewhat dilated (Figs. 27 and 28). Few or no synapses are found on the cell body. On the dendrites, however, synapses frequently occur, and several have an optic terminal as presynaptic element (Fig. 29). The initial part of the axon has bundles of microtubules and a subsurface coating.

Type XIV cells form in goldfish by far the most numerous population of tectal neurons (Meek and Schellart, '78). They have a cell body in layer 1 with a single apical dendritic process which runs via layers 2, 3 and 4 to layer 5. Dendrites are predominantly located in layer 5. The axon originates in layer 4/5 and may terminate in all tectal layers from 2 to 6. The examples studied in the EM had axon terminals in layer 3/4 and 4 (Fig. 17).

As described in the general layer composition, the cell bodies of type XIV neurons are closely packed and show gap-like junctions (Fig 30) They have a rounded nucleus with one nucleolus and only a small rim of cytoplasm (Fig. 30). Soma, dendritic shaft and dendrites make few synaptic contacts. Upon the dendrites in layer 5 (Figs 31 and 32) some optic nerve terminals were identified.

The axons studied form presynaptic elements with medium sized, round vesicles (Figs. 16b, 33 and 34) The distribution of synaptic contacts resembles that of type I axons. Only few occur on the descending part and between axonal swellings, and most contacts are found on the collaterals at thickened parts (Fig. 34).

Characteristics of three types of efferent neurons

The cells studied are presented in figure 37.

Type VI cells have a bipolar cell body in layer 5 and horizontally running dendrites in layer 6 and in layer 4/5 The axon arises in layer 4/5, becomes myelinated at some distance from its origin and leaves the tectum.

Cell bodies of type VI closely resemble cell bodies of type I, since they have the same shape, size and location, and the same cytoplasmic constituents (Fig. 35). However, their mitochondria have no electron dense matrix (cf. Fig. 35 with Fig. 18). Besides, they have more synapses on their soma and dendritic shafts than

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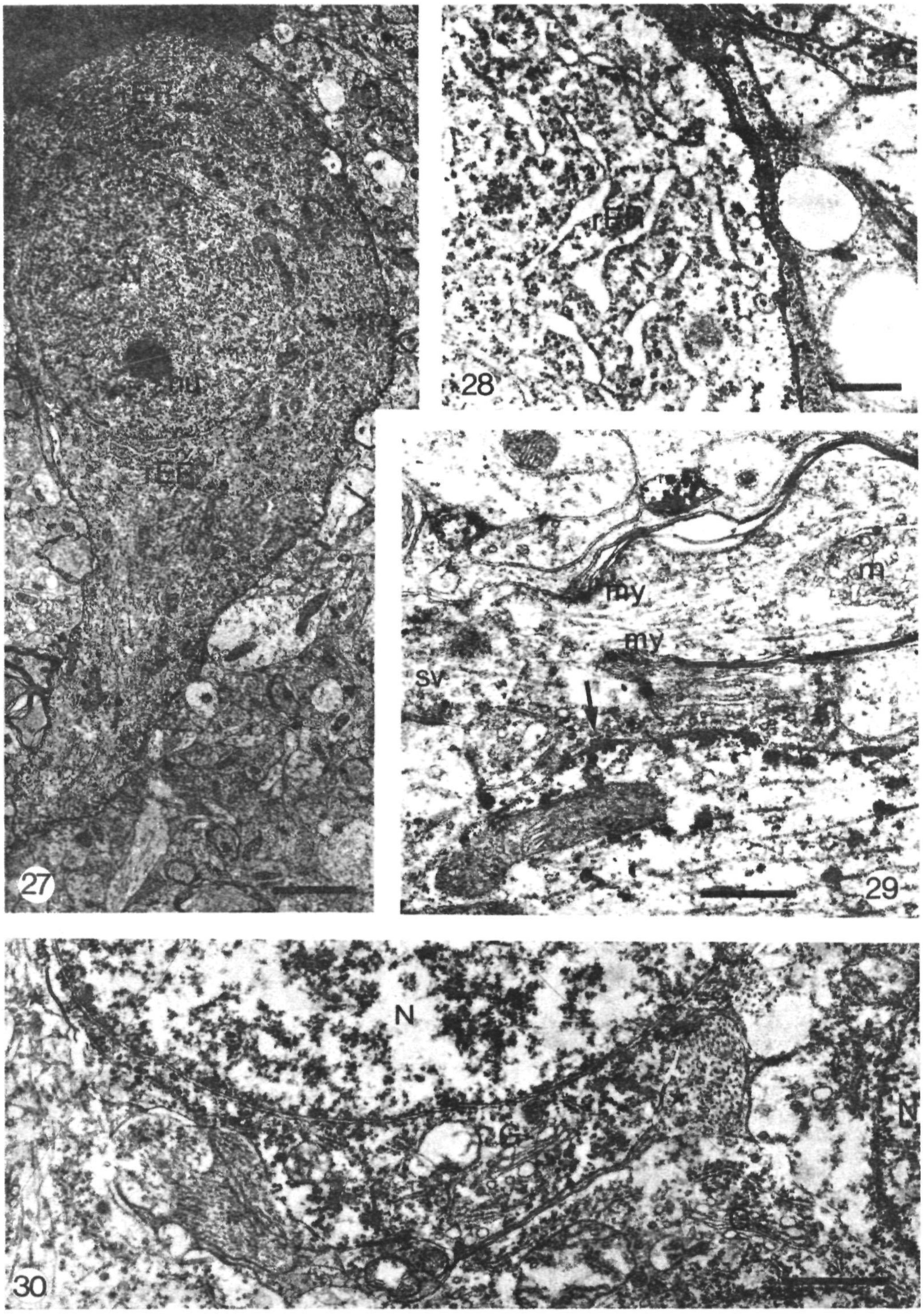
Fig 23 Apical dendrite in layer 7 of a type I cell (Fig 17 cell 1) with several spines making contacts with marginal axon terminals One terminal makes a contact on the dendritic shaft between spines (arrow) Calibration bar 1 μm

Fig 24 Basal dendritic shaft in layer 4 of a type I cell (Fig 17 cell 2) with a spine making synaptic contact Calibration bar 0 5 μm

Fig 25 Detail of the descending part of the axon of a type I cell (Fig 17 cell 3), showing three synaptic contacts with the type I axon as presynaptic element Calibration bar see Figure 26

Fig 26 Detail of an axon collateral of a type I cell (Fig 17 cell 3), showing a "bead-like structure", filled with synaptic vesicles and involved in a synaptic contact Calibration bar 0 5 μm


27
28
rER
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type I cells. Some of these synapses are formed by optic nerve terminals, one of which was observed to be involved in a triad (Fig. 36). On the dendrites in layer 6 and in layer 4/5 several types of synapses were distinguishable, but none was formed by an optic nerve terminal.

The axon origin clearly contains bundles of microtubules and a subsurface coating or "inner surface coating" (Figs. 40 and 41). Besides, it has a feature which is designated as an *"outer surface coating"* (Figs. 40 and 41). This consists of a grayish, amorphous material deposited against the axonal surface. It is not enclosed by membranes and is clearly located extracellularly. The "outer surface coating" stops where the myelin sheath starts and is interrupted at sites of synaptic contacts or apposition of glial elements. A surface coating is not specific for type VI, but present around all initial parts of myelinated axons studied in goldfish tectum. The same kind of material may be found at nodes of Ranvier in layer 6, 5 and 2, and unmyelinated axons may also show this feature at some parts. On the initial parts of the type VI axons only two synapses were observed with the axon as postsynaptic element. The axons of type VI cells do not form collaterals, which also holds for the other myelinated axons studied (type XII and XIII).

Type XII cells have a bipolar soma in layer 3 or 4 and dendritic trees in three regions: layer 5 to 5/6, layer 4 to 4/5 and layer 2/3. The myelinated axon arises from the apical dendritic shaft in layer 4 and has the shape of a shepherds crook.

The ultrastructural characteristics of the soma appear similar to those of type VI. Soma, dendritic shafts and dendrites are rather densely packed with synaptic contacts (Figs. 42 and 43), which only rarely occur on a spine-like structure (Fig. 42). Several types of presynaptic elements are observed, among which are some optic terminals in layer 5 and occasionally a terminal of an other type of tectal afferent in layer 2/3.

The initial part of the axon has an outer surface coating and may sometimes have a postsynaptic membranous specialization. In the hook of the "shepherds-crook" several dendrites wind around the axon. At this place the surface coating has disappeared, allowing a close apposition of these dendrites to the axon (Fig. 44).

Type XIII cells can be divided in type $XIII_1$ and $XIII_2$, based upon the course of the axon. This course is archiform for type $XIII_1$ and horizontal for type $XIII_2$ (Meek and Schellart, '78; Fig. 37). The remaining morphological characteristics are similar; they have a multipolar cell body in layer 2 and may have dendrites in four regions: layer 2, layer 3, layer 4/5 and layer 5/6. The dendrites in layers 5/6 and 4/5 were not impregnated in the cells used for the present study.

The cell body of *type* $XIII_1$ contains a nucleus with one nucleolus and abundant cytoplasm. It makes many synaptic contacts, and several times a tectal afferent has been identified as presynaptic element (Fig. 45). The dendrites have many contacts as well, but only one was observed with a tectal afferent (Fig. 46).

The axon of type $XIII_1$ is very peculiar. Its initial part deviates from that of other tectal axons in bearing a great number of synapses on its surface (Figs. 50 and 51). In the arch of all three archiform axons studied the myelin is interrupted, but an outer surface coating is present (Figs. 47 and 48). The axonal microtubules are concentrated at the inner side of the arch. At this side the axon is lacking surface coating material and has a very close apposition to a large number of thin profiles containing microtubules only (Fig. 48). These profiles appear to represent narrowed dendrites, that wind around the arch of type $XIII_1$ axons (Fig. 49). It is not clear to what kind of cells the narrowed dendrites belong.

The type $XIII_2$ cell studied has similar characteristics as type $XIII_1$, except that only few synapses were observed on its somatic as well as dendritic surface, among which one tectal afferent was observed. The initial part of its myelinated axons has no synapses.

DISCUSSION

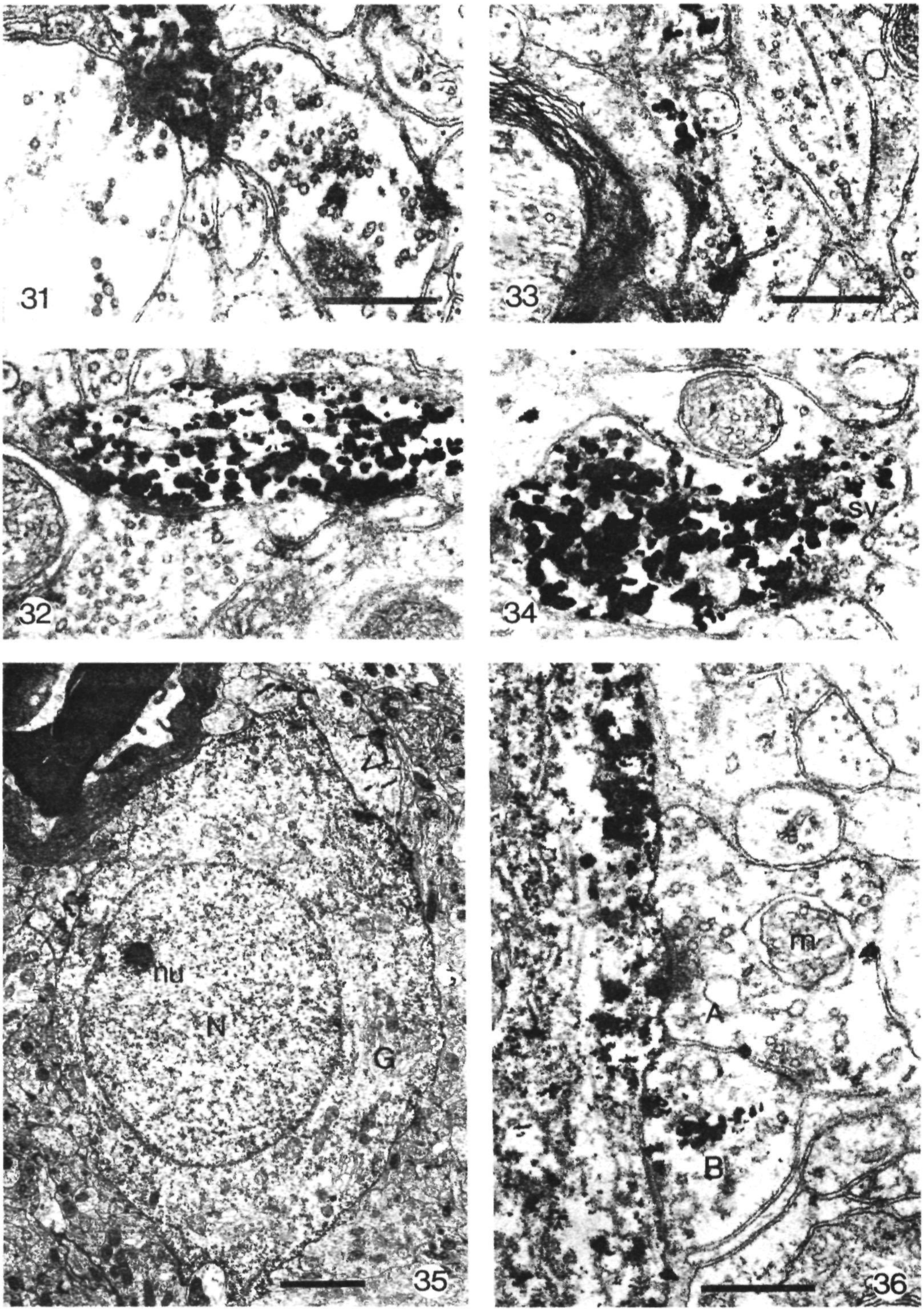
The Golgi-EM method of Fairén et al. ('77), originally worked out for the rat brain, combines the advantages of the light-microscopical

Fig. 27. Cell body of a type III cell (Fig. 17 cell 7). Calibration bar: 2 μm.

Fig. 28. Detail of the dilated rough endoplasmatic reticulum of a type III cell (Fig. 17 cell 6). Calibration bar: 0.5 μm.

Fig. 29. Optic nerve fiber, leaving its myelin sheath and making a contact (arrow) with the de-impregnated dendrite of a type III cell (Fig. 17 cell 7). Calibration bar: 0.5 μm.

Fig. 30. Part of the cell body of a de-impregnated type XIV cell (Fig. 17 cell 13), showing a synaptic contact (arrow) and junction-like membranous specializations (asterisk). Calibration bar: 1 μm.


31
33
32
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35
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A
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Fig 31 Dendrite of a type XIV cell (Fig 17 cell 8), making synaptic contact with two profiles containing round vesicles Calibration bar 0 5μm

Fig 32 Dendrite of a type XIV cell (Fig 17 cell 11), making synaptic contact with a profile containing pleomorphic vesicles. Calibration bar see Figure 31

Fig 33 Detail of the descending part of a type XIV axon (Fig 17 cell 9), involved in two synaptic contacts Calibration bar 0 5μm

Fig 34 Detail of an axon collateral of a type XIV cell (Fig 17 cell 9) with two synaptic contacts Calibration bar see Figure 33

Fig 35 Cell body of a de impregnated type VI cell (Fig 37 cell 15) Calibration bar 2μm

Fig 36 Part of the dendritic shaft of a type VI cell (Fig 37 cell 14) involved in a synaptic triade with the profiles A and B Profile A represents an optic terminal The vesicles in profile B are more obvious in adjacent sections However, the section presented shows the contact zones most clearly Calibration bar 0 5μm

identification of Golgi-impregnated cells with the precise electron–microscopical identification of their cellular components and synaptic contacts The present study reveals that this method is also applicable to the fish brain The fine structural details are satisfactory preserved after immersion prefixation with low concentrations of paraformaldehyde and glutaraldehyde (2% each) An improvement with respect to the preservation of fine structure as well as the prevention of the occurrence of impregnation artefacts can be obtained by using higher concentrations of these aldehyde (5% each) These higher concentrations -and consequently higher osmolarities- did not influence the size of vesicles and contact zones in our material

Although this paper is mainly devoted to the description of neuronal structures visualized with the Golgi-EM procedure, a number of terminals of tectal afferents were identified and analysed without impregnation The terminals from toral fibers in layer 7 closely resemble the corresponding structures in the carp (Ito, '70),

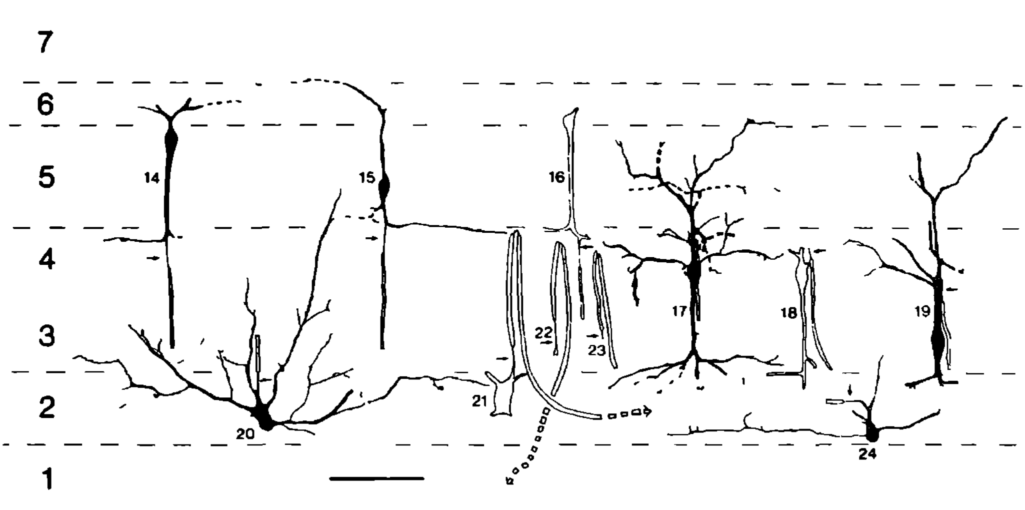
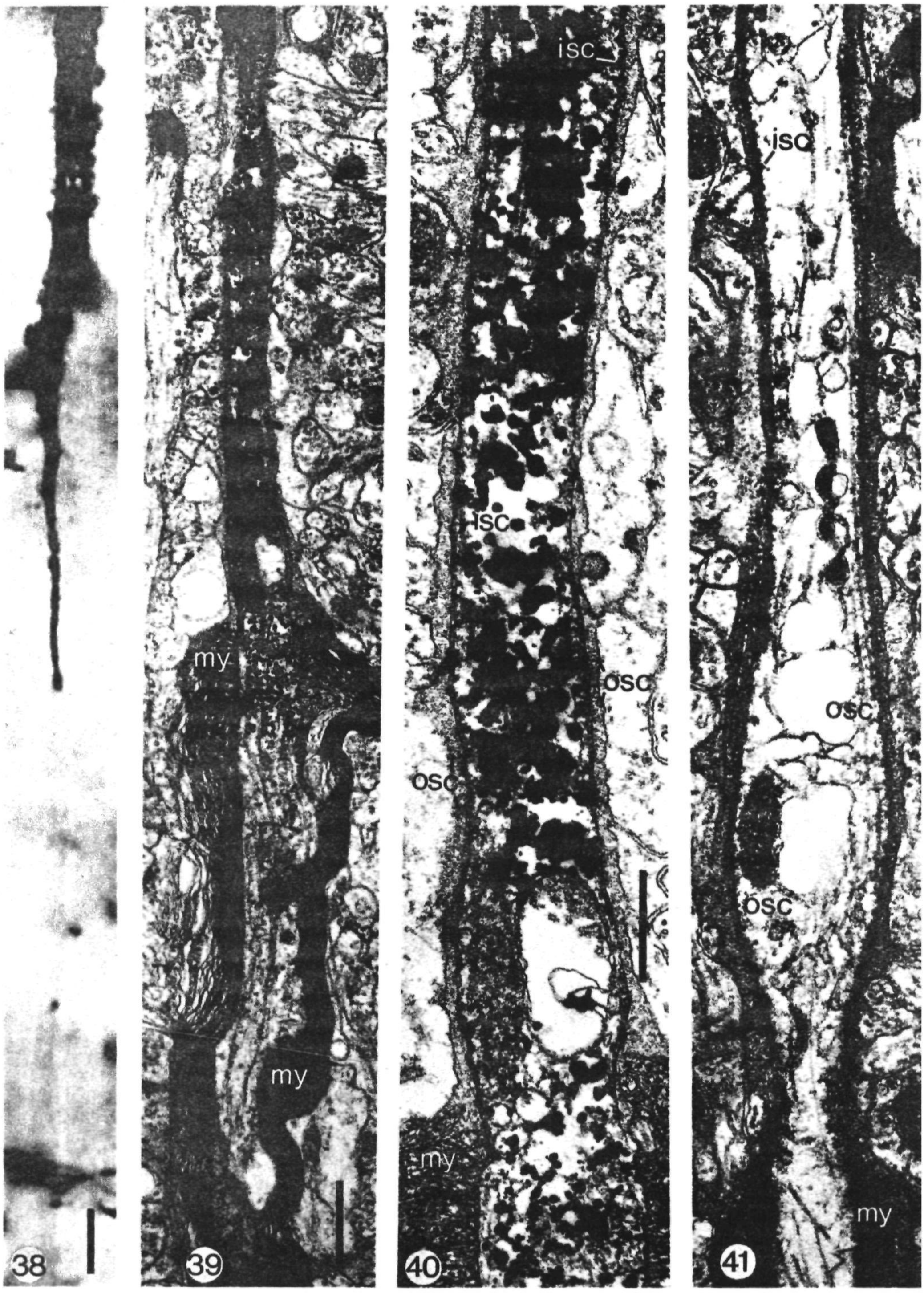


Fig 37 Composite drawing of the efferent neurons studied For explanation of the black, white and dotted cellular elements, see Figure 17 The numbers indicate 14-16, type VI cells, 17 19, type XII cells, 20 and 21, type XIII$_1$ cells 22 and 23, axons of type XIII$_1$ cells and 24, a type XIII$_2$ cell Arrows indicate axons The calibration bar 100μm


isc
isc
osc
osc
my
my
my
isc
osc
osc
my
38
39
40
41

E. plumieri (Laufer and Vanegas, '74a) and *H. rufus* (Ito et al., '80). The vesicles in these terminals are of the same size in *E. plumieri* (about 40 nm) and the goldfish (35-52 nm, mean value 43 nm), but appear to be smaller in *H. rufus* (25-40 nm). Terminals with flat vesicles, as occur in the *marginal layer* of *H. rufus*, were not observed in the present study, nor in *E. plumieri* (Laufer and Vanegas, '74a) or the carp (Ito, '70).

Optic nerve terminals were identified in the present study by their characteristic pale mitochondria and large round vesicles. The degeneration experiments of Laufer and Vanegas ('74b), Airhart and Kriebel ('80) and Ito et al ('80) provide a firm basis for this identification in teleosts. Pale mitochondria with dilated cristae even seem to be a general characteristic of retino-tectal fibers in vertebrates (for discussion, see e.g. Laufer and Vanegas, '74a; Vrensen and de Groot, '77). The vesicles in optic terminals are of the same size in the teleosts investigated (goldfish: 40-60 nm, mean value 49 nm; *E. plumieri:* 40-60 nm (Laufer and Vanegas '74a) and *H. rufus:* 30-60 nm (Ito et al., '80). Axo-axonal contacts of optic nerve terminals, as described for goldfish tectum in the present study, were also described by Ito ('70) for carp tectum and by Ito et al. ('80) for *H. rufus*, but were not observed in *E. plumieri*.

Apart from the identifiable optic nerve fibers, myelinated axons of unknown origin also terminate in layer 5. These most probably represent nonretinal tectal afferents because (1) they also frequently occur in layer 2 and 3, where optic fibers only rarely terminate (Sharma, '72a; Grafstein, '67; Neale et al., '72; Marotte and Mark, '75; Schmidt, '79); (2) in teleosts, only terminals with pale mitochondria have been found to degenerate after eye enucleation (Laufer and Vanegas, '74b; Airhart and Kriebel, '80; Ito et al., '80); and (3) degeneration in layer 5 as well as in layers 2 and 3 is found after interruption of various non-visual afferent systems (Marotte and Mark, '75; Ito et al., '80).

The frequent occurrence of the non-optic afferents in layers 2 and 3 indicates that these layers are, beside layer 5 and layer 7, a third main region of tectal input. The fact that most afferents in layer 2 and 3 contain pleomorphic vesicles may indicate that their influence is predominantly inhibitory, in contrast to visual and toral afferents, which contain round vesicles and are thought to be excitatory.

Six cell types were studied by means of the combined Golgi-EM method. Without exception, all structures identified in the light microscope as dendrites appeared to be postsynaptic and nerver showed any presynaptic regions. The same holds for neuronal cell bodies, and also for the initial parts of myelinated axons. Consequently, these components must be considered as exclusively postsynaptic structures. In contrast, the axons of the interneurons studied (type I and XIV) are exclusively presynaptic. This also holds for the tectal afferents studied. These observations suggest that in goldfish, so far as the cell types studied are concerned, tectal synaptology is characterized by straight-forward synapses (axo-dendritic, axosomatic and -for initial parts of myelinated axons only- axo-axonal). Even careful investigation never revealed other types of synapses for the six cell types studied (e.g. dendro-axonal or dendro-dendritic). However, the few contacts between optic terminals and small structures of unknown origin containing vesicles form an exception to this rule.

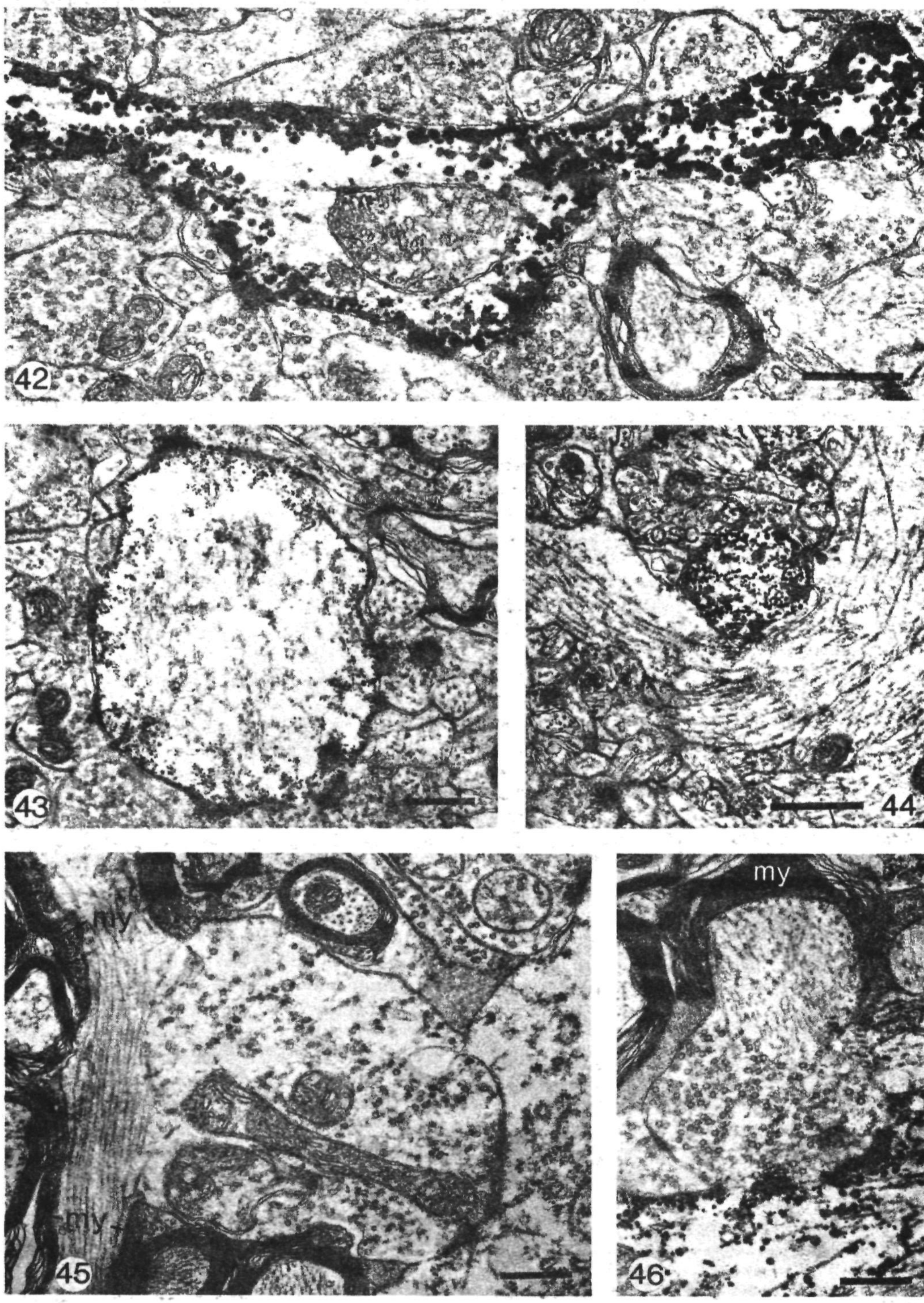
The synaptology of the tectum of *Holocentrus rufus*, which was recently investigated by Ito et al. ('80) appears to be more complex in several respects. For instance, presynaptic dendrites (the F_2 type of terminal described by Ito et al., '80) are not observed in the goldfish, and equally no glomerular arrangement of synapses, as described for the S_3 type of terminal in *H. rufus*, could be discerned in the goldfish tectum. These and other smaller differences, e.g. the absence in goldfish of an F_1 type of terminal in the *marginal layer* and the absence of dendritic spines in the *periventricular gray layer*, might well reflect the high visual specialization of *Holocentrus* in comparison to the goldfish (Schroeder et al., '80).

Fig. 38. Light microscopic photograph of the axon of a de-impregnated type VI neuron (Fig. 37 cell 14). Calibration bar: 5 μm.

Fig. 39. Electron micrograph of the same axon as shown in Figure 38. This picture shows that the impregnation stops where the myelin sheath starts. Calibration bar: 1 μm.

Fig. 40. Detail of a neighbouring serial section of Figure 39, showing the inner surface coating (isc) and the outer surface coating (osc). Calibration bar: 0.5 μm.

Fig. 41. Detail of the axon of an unimpregnated type VI cell (Fig. 37 cell 16), which shows the same features as the de-impregnated example of Figure 40. Calibration bar: see Figure 40.


42
43
osc
44
my
my
45
my
46

Some cell types appear to have characteristic ultrastructural features, which, once established by means of the Golgi-EM method, allow subsequent identification even without impregnation. For example, the dark mitochondria of type I neurons are so characteristic that this cell type can easily be distinguished from type VI neurons, which in Golgi preparations they closely resemble. Moreover, the cytoplasm of type I cells darkens after fixation in 2% paraformaldehyde / 2% glutaraldehyde, a feature not observed in any other type. Type III cells are characterized by their dilated rough endoplasmic reticulum, so that they can be clearly distinguished from superficially located type VI neurons.

The initial parts of myelinated axons appear to have the same characteristics as in the mammalian brain: bundles of microtubules and an inner surface coating (the subsurface coating of Peters et al., '76). However, in goldfish tectum they also have a so called *"outer surface coating"*, which, incidentally, may also be observed around unmyelinated axons. The axonal outer surface coating was also observed in the tectum of the carp (Meek, unpublished results). The origin and composition of this extracellular material are unknown; a special relation with glial elements was not observed. Its presence might be related to the experimental difficulties in obtaining extracellular recordings of action potentials of intrinsic tectal cells (Schellart and Riemslag, pers. comm.).

Several archiform axons of type $XIII_1$ neurons were studied, revealing a puzzling relation with looping and narrowing dendrites of unknown origin. The interruption of the myelin sheath, the absence of an outer surface coating at the inside bend of the arch and the close apposition to the looping dendrites strongly suggest a functional significance. Conceivably, the axon might influence the dendrites as a feed-back mechanism, or the dendrites might influence the axon, in an excitatory way, e.g. in fast, reflex stimulation, or in an inhibitory way, e.g. to block spike propagation. In this respect it should be noted that the same type of axo-dendritic apposition is observed for type XII axons, although for these it is less pronounced.

The axonal terminations of two types of interneurons (type I and XIV) were studied. Both contain round vesicles with a mean size of 45 or 46 nm. Most probably the axon terminals of type I cells are included in the S_4 type of terminal described for *H. rufus* by Ito et al. ('80), since the size of the terminals and the shape and size of the synaptic vesicles are quite comparable (25-70 nm in *H. rufus;* 35-60 nm, mean value 45 nm in goldfish). In particular, the dense band of S_4 terminals in layer 3/4, which do not degenerate after telencephalic lesions, might well represent the axons of type I or pyramidal cells in *H. rufus*. Considering the high frequency of occurrence, the size of terminals, the types of postsynaptic elements and the presence of small, round vesicles, the axon terminals of type XIV or periventricular cells are most probably included in the S_5 type of terminal described in *H. rufus* (Ito et al., '80). However, the size of vesicles in the S_5 terminals is smaller in *H. rufus* (25-40 nm) than in type XIV axon terminals in goldfish (35-55 nm, mean value 46 nm); a similar discrepancy as found for the S_1 terminal in *H. rufus* and the comparable type A terminal in goldfish (values being 25-40 nm and 35-52 nm respectively). The reason for this difference is not clear, but does not seem to be methodological, since the sizes of other comparable terminals, e.g. of type S_2 and type B (the optic terminals), and of type S_4 and type I axon terminals, appear to be similar.

Since type XIV is by far the most frequently occurring category of cells (Meek and Schellart, '78), with axons containing round synaptic vesicles and making a substantial number of synaptic contacts (Meek, '81), one should expect that most presynaptic elements in goldfish tectum contain round vesicles. However, a general survey shows that according to our criteria at least half of all terminals contain vesicles which are pleomorphic. On this ground one should assume that a substantial portion of the type XIV cells has terminals with pleomorphic

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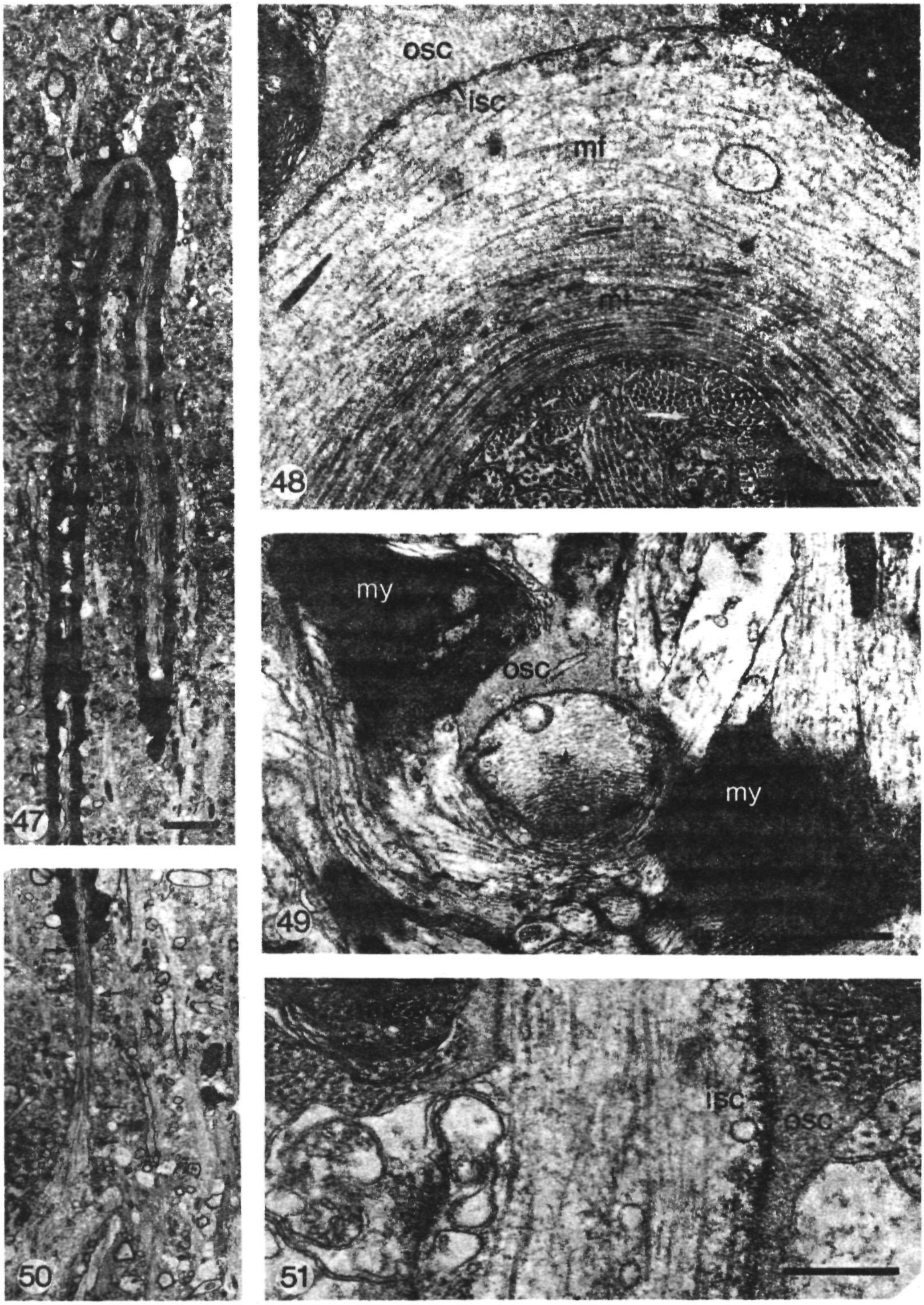
Fig. 42. Dendrite in layer 4 of a type XII cell (Fig. 37 cell 17), with several synaptic contacts. Calibration bar: 0.5 μm.

Fig. 43. The apical dendritic shaft of a type XII cell (Fig. 37 cell 17), densely packed with synapses. Calibration bar: 0.5 μm.

Fig. 44. Transverse section through the "hook" of the axon of a type XII cell (Fig. 37 cell 17) showing a close apposition to looping dendrites. Calibration bar: 1 μm.

Fig. 45. Axon terminal of type E, terminating *en passant* on the cell body of an unimpregnated type $XIII_1$ cell (Fig. 37 cell 21). Calibration bar: 0.5 μm.

Fig. 46. Axon terminal of type C, terminating on a dendrite of a de-impregnated type $XIII_1$ cell (Fig. 37 cell 20). Calibration bar: 0.5 μm.


47
osc
isc
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mt
48
my
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my
49
50
isc
osc
51

vesicles. In many respects, type XIV cells form a heterogeneous population, and the two axons studied in this paper are too few to exclude this possibility. Another possibility is that Golgi impregnation, although it does not alter the size and shape of round vesicles (see METHODS), does cause a rounding of pleomorphic vesicles. Impregnated boutons clearly showing flattened vesicles were not observed in goldfish tectum, and equally were not described by others (Fairén et al., '77; White, '78; Peters et al., '79; Peters and Proskauer, '80; Difiglia et al., '80).

To unravel tectal circuitry, it is necessary to identify both sides of a synapse. In the present study this was occasionally possible, viz. when impregnated dendrites or cell bodies make a contact with optic nerve terminals or terminals sprouting from other myelinated axons. Based on these findings it can be concluded that all cell types studied with postsynaptic components in layer 5 (types I, III, VI, XII and XIV) receive some degree of visual input, and that the basal dendrites of type XII and the soma and basal dendrites of type XIII receive a certain amount of input from non-visual tectal afferents. The conclusions concerning tectal circuitry are summarized in figure 52. In the next paper (Meek, '81) the synaptic contacts of the cell types studied will be characterized more quantitatively.

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Fig. 47. The archiform axon of a type $XIII_1$ cell (Fig 37 cell 21) at low magnification, showing the interruption of the myelin sheath in the arch of the axon Calibration bar 5 μm.

Fig. 48 Detail of a neighbouring serial section of Figure 47, showing the arch of the axon in close apposition to the narrow dendritic profiles at the inside bend Calibration bar 0.5 μm.

Fig 49 Oblique section through the unmyelinated arch (asterisks) of a type $XIII_1$ axon (Fig 37 cell 22), showing the looping and narrowing of some dendrites that are in close apposition to the axon Calibration bar 1 μm

Fig 50. The initial part of the same axon as shown in Figure 47 Arrows indicate synaptic contacts For calibration bar. see Figure 47

Fig. 51. Detail of a neighbouring serial section of Figure 50, showing an axo-axonal synaptic contact Calibration bar. 0 5/um.

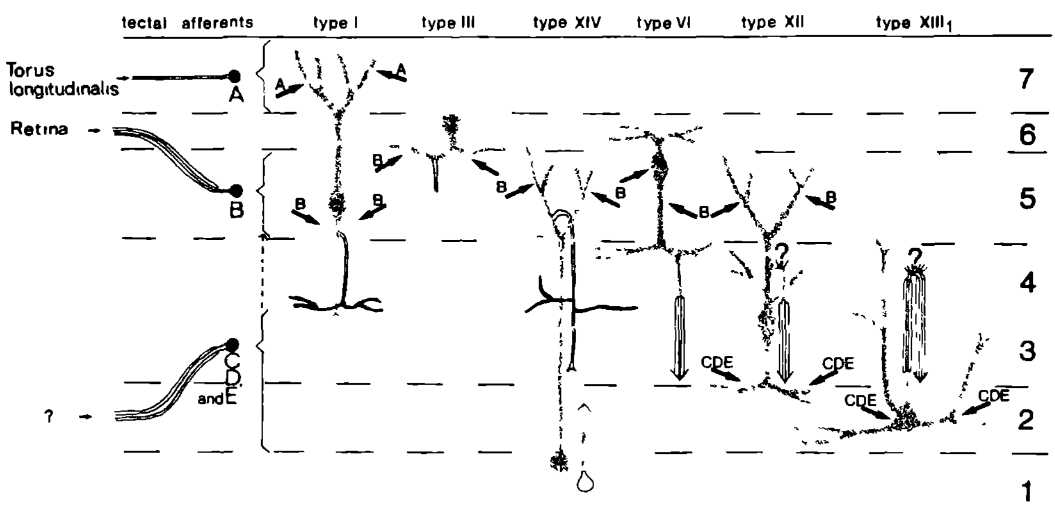

Fig 52 Schematic drawing, summarizing the results concerning tectal circuitry *Black* indicates presynaptic structures, *Gray* indicates postsynaptic structures and *White* structures are not involved in synaptic contacts Arrows indicate synaptic contacts of identified presynaptic elements (see Fig 16) with the cell types studied.

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CHAPTER IVb

A Golgi-Electron Microscopic Study of Goldfish Optic Tectum. II. Quantitative Aspects of Synaptic Organization

J MEEK
Department of Morphology, The Netherlands Ophthalmic Research Institute, P O. Box 6411, 1005 EK Amsterdam, The Netherlands

ABSTRACT The size, density, and number of the synaptic contacts of three types of interneurons (types I, III, and XIV of Meek and Schellart, '78) and three types of efferent neurons (types VI, XII, and XIII) of the goldfish optic tectum were quantified by means of a quantitative stereological study of Golgi-EM serial sections. Furthermore, an estimation was made of the percentage of optic terminals on these six cell types and of the ratio between terminals with pleomorphic and terminals with round vesicles.

The mean density of contacts per receptive component (i.e. the cell body and the different parts of the dendritic tree) varies from 0 to 100 per 100/μm^2 surface, corresponding to 0–8% receptive surface. Each cell type has a characteristic average density as well as a characteristic density distribution along the distinct components. This suggests that the receptive components of the tectal cell types investigated have a predetermined density and that a morphological classification of tectal cells has functional relevance.

The mean length of the contact zones in the ultrathin sections varies from 213 to 332 nm for identified postsynaptic elements and from 188 to 293 nm for identified presynaptic elements. The size of the contacts on the distinct receptive components appears to be primarily related to the tectal lamination pattern. Distinct types of axons, however, have characteristic mean sizes of contacts. This might suggest that the size of the contacts, contrary to their density, is primarily determined by the presynaptic elements.

The mean number of synaptic contacts calculated per cell type is as follows: type XIV, 200; type III, 450; type VI, 1,400; type I, 2,100; type XII, 4,200; and type $XIII_1$, 5,400. Multiplication of these numbers with the number of cells per tectal half shows that the population of type XIV cells has by far the most synaptic contacts, since their low number of synaptic contacts is clearly overruled by their high frequency of occurrence.

Optic terminals, identified by their characteristic mitochondria and large round vesicles, appear to contribute to about 10–20% of the contacts on identified postsynaptic elements in layer 5. The ratio between presynaptic elements with pleomorphic vesicles and those with round vesicles shows a slight tendency to increase when the distance to the origin of the axon decreases.

It is concluded that a combination of the Golgi-EM technique with quantitative stereological methods appears well suited to the study of the synaptic organization of brain centers, and that combination of quantitative Golgi-EM with neuronal tracing methods (degeneration, HRP, autoradiography) offers good prospects for detailed investigations of neuronal connectivity.

The midbrain roof of the goldfish has been the object of many physiological investigations (e.g. Jacobson and Gaze, '64; Landreth et al., '75; Cook and Horder, '77; Schellart et al., '79), and its cytoarchitecture is quite well known (Leghissa, '55; Meek and Schellart, '78; Romeski and Sharma, '79). However, data on

Address reprint requests to J Meek, Department of Anatomy, University of Nijmegen, Postbox 9101, Geert Grooteplein N 21, 6500 HB Nijmegen, The Netherlands

the ultrastructure of the goldfish tectum are lacking. Therefore, the ultrastructure and synaptology of six of the most common cell types were described in the preceding paper (Meek, '81), using the combined Golgi-EM technique of Fairén et al. ('77). The present paper deals with the quantitative aspects of the synaptology of these six cell types of the goldfish tectum. The study has been performed to investigate the synaptic relevance of the classification of Meek and Schellart ('78) and to gain a detailed insight into the synaptic organization of the cell types selected. Several stereological methods have been adapted and applied for this purpose.

For the dendrites and somas of the cell types selected, the size, density, and number of the synaptic contact zones are determined and the number of optic nerve endings per cell type is estimated. Furthermore, the ratio between presynaptic elements with pleomorphic and with round vesicles is estimated for the different components of the cell types. For the axons the size and number of the contacts are presented.

METHODS

Measurements

The quantitative analysis was carried out on the cells previously selected for a description of their ultrastructure (Meek, '81). These cells are presented in Figure 1. The method used for deimpregnation and reconstruction has been described in detail in the preceding paper (Meek, '81) and will here be summarized only briefly. Tecta are prefixed with aldehydes and impregnated by the rapid-Golgi procedure. Next, 50 μm sections are cut on a Vibratome, gold-toned, and deimpregnated (according to Fairén et al., '77). After embedding in Epon 812, sections containing interesting cells are drawn, photographed, and mounted for thin sectioning. Serial thin sections are cut and each series of ten consecutive sections is mounted on one grid. The deimpregnated cells, and sometimes unimpregnated cells present in the sections, are reconstructed by means of electron micrographs at low magnification.

For the present quantitative analysis, basically one out of the ten serial sections was selected from each grid. Preferably a single complete section, not interrupted by grid bars, was selected. If this was not possible, two or more sections with complementary parts were chosen. The complete somatic, dendritic, and axonal surface membrane of the neuron of interest present in these sections was photographed. The final magnification of the photographs was about 30,000 × (the exact magnification was determined using a grating replica). Since ten serial sections were collected on each grid, about 10% of the total membrane of the cells investigated was photographed and subsequently analyzed. The cells studied in this way are drawn in white or black in Figure 1.

On each photograph three parameters—i.e. the number of contacts, the length of each contact, and the length of the membrane trace—were measured (Fig. 2), using a semiautomatic measuring device (MOP / AMO1). In a second stage the data for homologous components of one cell were added together (Fig. 3b), and in a third stage homologous components of all cells of a specific cell type were put together (Fig. 3c). In the present paper the term *component* refers to distinguishable parts of a neuron, i.e. the cell body, different parts of the dendritic shaft and tree and different parts of the axon. The different receptive or postsynaptic components that are distinguished per cell type are indicated in Table 1.

In this way the following values became available, per cell as well as per cell type:

B: The total length of the neuronal membrane trace measured.

N_A: The number of contact zones counted.

$\bar{L}$: The length of the contact zones.

The frequency distribution of L is also obtained in this way.

For characterization of the presynaptic elements on identified dendrites and cell bodies, the following approaches were applied. To determine the percentage of optic terminals that make contact with a certain cell type, in layers 5 and 6 the occurrence of the following five groups of presynaptic elements was determined per cell type.

1: *Optic nerve terminals* (with large round vesicles and characteristic pale mitochondria with dilated cristae; Meek, '81).
2: *Possible optic nerve terminals* (with large round vesicles but without mitochondria).
3: *Nonoptic terminals with large round vesicles* (distinguished from group 1 by their different mitochondria).
4: *Other nonoptic terminals* (with pleomorphic vesicles or with small round vesicles).
5: *Unclassified terminals* (with only few vesicles and without mitochondria).

Furthermore, the number of presynaptic elements with round and with pleomorphic vesicles was determined per component of each cell type. This was done by means of a subjective

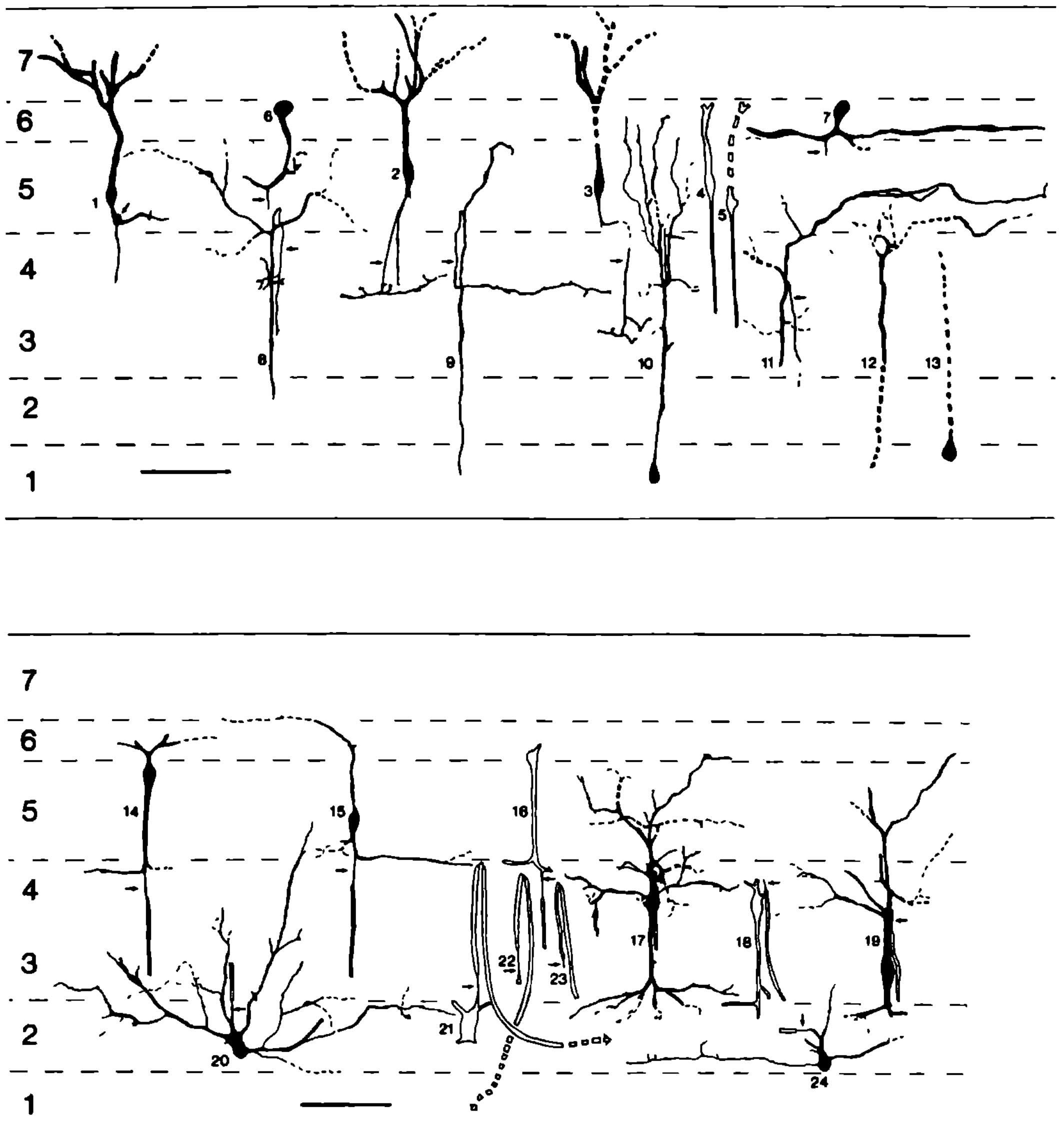


Fig 1. Composite drawing of the neurons studied in this paper Black indicates those parts of the deimpregnated neurons that were studied in the EM, dotted parts represent deimpregnated structures that were not, and white parts represent unimpregnated structures that were studied in the EM The numbers indicate. 1–5, type I cells, 6 and 7, type III cells; 8–13, type XIV cells, 14–16, type VI cells; 17–19, type XII cells, 20 and 21, type $XIII_1$ cells, 22 and 23, axons of type $XIII_1$ cells, 24, a type $XIII_2$ cell The classification with Roman numerals is according to Meek and Schellart ('78) Arrows indicate axons. The calibration bar is 100 μm

classification, for which only terminals with more than 20 vesicles were used. A terminal was classified as "round" when all vesicles were round, otherwise it was classified as "pleomorphic."

Calculations

For the calculations, the contact zones were considered as randomly sectioned, flat, circular disks (for a discussion on the shape of contact zones, see Vrensen et al., '80). The quantities

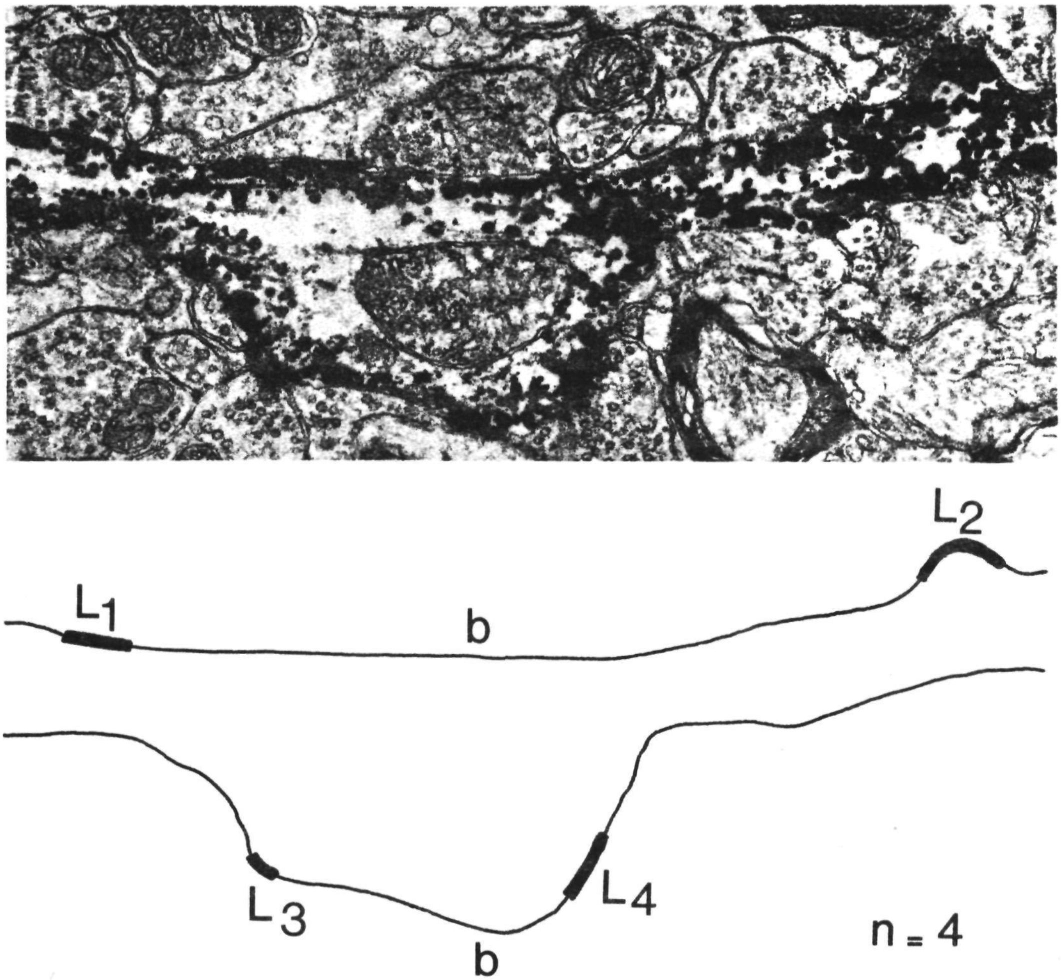


Fig. 2. The parameters measured on each photograph. n = number of contacts; L = length of each contact; b = length of the membrane trace. A part of the membrane trace is considered as a contact zone when it clearly shows synaptic membranous specialization associated with presynaptic vesicles.

used in the next paragraphs are defined in the list of abbreviations.

The size of contacts. Under the assumptions just enumerated, the mean surface area, the mean diameter ($\overline{\triangle}$) and the mean chord length ($\overline{L}$) of a population of contacts are proportional to each other and thus interchangeable. Since for thin sections in general and consequently also for the Golgi-EM material used in this study only L can be measured, this value was chosen to express the mean size of the contacts. The distribution of L (See Fig. 3) was used for statistics.

For the samples for which a sufficient amount of measurements was available, the *distribution* of the diameters of the contacts was derived from the *distribution* of L, according to the method given by Weibel and Bolender ('73; p. 252). From this it appeared that $\overline{\triangle} = 1.2 \times \overline{L}$, instead of the theoretically expected relation $\overline{\triangle} = 1.27 \times \overline{L}$ ($= \frac{4}{\pi} \times L$; Weibel and Bolender, '73). This is due to the fact that the most tangential section of a contact (cap section) is not recognizable in EM sections. Consequently for the following calculations the relation $\overline{\triangle} = 1.2 \times \overline{L}$ will be applied.

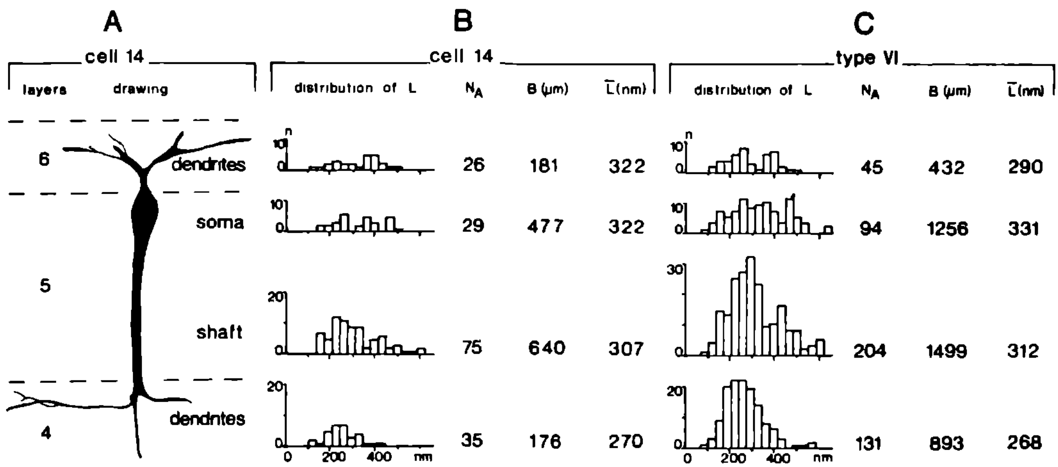


Fig 3 The procedure applied to process the measurements, illustrated for type VI A) Drawing of one of the type VI cells studied (cell 14 of Fig 1) B) The values obtained for the cell drawn under A C) Addition of the values obtained for all three type VI cells studied

Table 1 Receptive components in the goldfish tectum and some quantitative parameters regarding their synaptic density

Type	Receptive component	N_A	B (μm)	Density	Density per cell[1]					
I	dendrites in layer 7	362	2,720	38	40	34	42			
	dendrites in layer 7[2]		(1,679)	(61)	(72)	(49)	(59)			
	apical dendritic shaft	28	1,780	5	4	6	8	4		
	cell body	17	870	6	7	1	9	6	0	
	dendrites in layer 5	20	258	23	23					
	basal dendritic shaft[3]	50	770	18	22	21	12	12	45	
	dendrites in layer 4-3/4	54	542	31	21	32		68	27	
III	cell body	5	808	4	2	6				
	dendrites in layer 5	131	1,769	21	20	22				
XIV	dendrites in layer 5	136	2,510	19	33	24	11	24	40	
	dendritic shaft	70	2,026	14	36	6	0	9	36	
	cell body (superficial)[4]	12	446	6						7
	cell body (deep)[4]	0	365	0			0			
VI	dendrites in layer 6	45	432	30	37	26				
	cell body	94	1,256	19	16	16	27			
	dendritic shaft	204	1,499	36	32	24	51			
	dendrites in layer 4/5	131	893	46	61	41	47			
XII	dendrites in layer 5	322	1,297	67	65	74				
	apical dendritic shaft	161	743	69	72	68	66			
	dendrites in layer 4	379	1,168	103	96	123	119			
	cell body	191	922	70	67	71	77			
	basal dendritic shaft	256	874	88	80		96			
	dendrites in layer 2/3	433	1,581	84	79	108	87			
$XIII_1$	dendrites in layer 4-3/4	208	809	78	78					
	dendrites in layer 3-2	732	2,995	71	71	92				
	cell body	307	1,203	64	50	68				
$XIII_2$	dendrites in layer 3-2	69	938	24	24					
	cell body	13	368	11	11					

[1]The cells are presented in the same sequence as in Figure 1
[2]Values obtained leaving the membrane trace of the spines out of account
[3]The part between the cell body and the axon origin
[4]Beside the impregnated examples, also ten unimpregnated profiles were taken into account

Abbreviations	
b	length of the neuronal membrane trace in one micrograph
B	length of the total neuronal membrane trace of a distinct neuronal component in all sections sampled
$\overline{D}$	mean caliper diameter
$\overline{\triangle}$	mean diameter
h	minimal height of a tangential section (cap section) of a circular disk, necessary for recognition in an ultrathin section
L	length of a contact zone as seen in an ultrathin section
M	length of a structure, projected on a plane parallel to the direction of the section
n_a	number of contacts counted on one micrograph
N_A	total number of contacts of a distinct neuronal component as counted in all sections sampled
$N_{a(x)}$	number of contacts belonging to a certain class (class x), as counted in the sections sampled
N_V	number of contacts in a reference volume
S_V	surface of a component in a reference volume
T	section thickness
V	reference volume

The density of contacts per surface area. A general formula is N_V/S_V (Mayhew, '79). N_V represents the number of contacts in a reference volume; S_V represents the surface area of a neuron in a reference volume.

$$N_V = \frac{N_A}{\overline{D} + T - 2h} \quad \text{(Weibel and Bolender, '73)} = \frac{N_A}{\overline{D}} \quad (\text{for our material,} \quad h \simeq \tfrac{1}{2}T)$$[2]

For thin discs $\overline{D} = \frac{\pi}{4} \times \overline{\triangle}$ (Hilliard, '67) $= \frac{\pi}{4} \times 1.2 \times \overline{L}$ (see size of contacts). $S_V = \frac{4}{\pi} \times B$ (see Mayhew, '79; $\frac{4}{\pi}$ is the reverse of the caliper factor of Hilliard, '67).

$$\text{So, } N_V / S_V = \frac{N_A}{\frac{\pi}{4} \cdot 1.2 \cdot \overline{L} \cdot \frac{4}{\pi} \cdot B} = \frac{N_A}{\overline{L} \cdot B} \cdot \frac{1}{1.2} \qquad (1)$$

By means of this formula the density, expressed per μm^2 surface, can be calculated from the parameters that were measured. In the Results section the values obtained by this formula will be multiplied by factor 100 to avoid fractions. The values consequently represent the number of synaptic contacts per 100 μm^2 surface area.

The percentage receptive surface. This is given by

$$\frac{\overline{L} \cdot N_A}{B} \cdot 100\% \qquad (2)$$

The number of contacts per component measured. This is basically obtained by multiplication of the density of contacts by the total surface of a neuronal component (density × surface). The formula for the density is given above. The surface of a component is $B \times T \times \frac{4}{\pi} \times 10$ ($\frac{4}{\pi}$ is the reverse caliperfactor (Hilliard, '67); the factor 10 comes from the fact that 10% of all sections of each component was measured).

Consequently the number of synapses per component measured (N_{comp}) is

$$N_{comp} = \text{density} \times \text{surface} = \frac{N_A}{\overline{L} \cdot B} \cdot \frac{1}{1.2} \cdot B \cdot T \cdot \frac{4}{\pi} \cdot 10 = \frac{N_A \cdot T}{\overline{L}} \cdot \frac{33}{\pi} \qquad (3)$$

For the section thickness (T) a value of 75 nm was chosen, since sections with a white interference color were cut.

The mean number of contacts on a specific component of a certain cell type (N_{type}). For cell bodies, the values obtained for the specimens measured were averaged. For dendrites, an estimation was obtained by application of the following formula:

$$N_{type} = N_{comp} \cdot \frac{\overline{M}_{type}}{M_{measured}} = \frac{N_A \cdot T}{\overline{L}} \cdot \frac{33}{\pi} \cdot \frac{\overline{M}_{type}}{M_{measured}} \qquad (4)$$

$M_{measured}$ represents the total length of all homologous dendrites measured, projected on the plane of sectioning. $\overline{M}_{type}$ represents the mean length of the homologous dendrites under investigation per cell type, projected on the plane of sectioning. Values for $\overline{M}_{type}$ were obtained from material used for a previous Golgi study (Meek and Schellart, '78).

In the Results section N_{type} is presented as the number of synapses. It should be noted that the formula applied does not correct for variations in the mean angle between the dendrites and the plane of sectioning, nor for variations in thickness of the dendrites. For large samples these variations approach constant mean values, but in small samples (e.g., $M_{measured}$), some

[2]T is about 75 nm (white interference colour) h is estimated at 30–40 nm, derived from our findings that $\overline{\triangle}$ is about 350 nm (Fig 5) and that the smallest value measured for L are about 150 nm or more, which means that cap sections thinner than about 30–40 nm are missed

variations may be expected, which cause deviations in the estimates obtained.

The percentage of contacts with optic terminals per component. As explained under *measurements*, the frequency of occurrence of five groups of presynaptic elements, indicated 1–5, was counted. An estimation of the *minimal value* is obtained when only the positively identified optic terminals (1) are compared with the total number of contacts counted per component:

$$\frac{N_{A(1)}}{N_A} \cdot 100\% \qquad (5)$$

An estimation of the *maximal value* is obtained when all terminals in group 1 and 2 are considered as optic terminals and when the unclassified terminals (5) are assumed to have the same distribution as the classified terminals (1–4):

$$\frac{N_{A(1)} + N_{A(2)}}{N_A - N_{A(5)}} \cdot 100\% \qquad (6)$$

The most reasonable value is obtained under the assumptions that in group 2 the ratio between optic and nonoptic terminals is equal to $N_{A(1)}/N_{A(3)}$ and that the unclassified terminals (group 5) have the same distribution as the classifiable ones:

$$\frac{N_{A(1)}+N_{A(2)} \dfrac{N_{A(1)}}{N_{A(3)}+N_{A(1)}}}{N_A - N_{A(5)}} \cdot 100\% \qquad (7)$$

The *number of optic terminals per component* is easily derived from the percentage of optic terminals and the total number of contacts (N_{type}).

The ratio between terminals with pleomorphic vesicles and terminals with round vesicles. For this $N_{A\ (pleomorphic)}$ was divided by $N_{A\ (round)}$, which implies the assumption that unclassifiable terminals have a distribution similar to the classifiable terminals.

Statistics. Differences in distribution of the length of the contact zones in the sections (L) were tested with the chi-square test for two samples. Differences in density of contacts were tested with the Mann-Whitney test (Siegel, '56).

RESULTS

The morphological characteristics of the cells investigated are presented in Figure 1. In this figure the tectal layers are also indicated. For a detailed description of the tectal cell types the reader is referred to Meek and Schellart ('78). The different receptive components distinguished in the present study are indicated in Table 1 (first column) and schematically drawn in Figure 4a. The present results regarding the size, density, and number of contacts, the percentage of receptive surface, the percentage and number of optic terminals, and the ratio between terminals with pleomorphic and with round vesicles are summarized in Figure 4. In the next paragraphs some comments on these results will be made.

The size of the contacts

Comparison of the mean size of synaptic contacts on the various cellular components reveal that no gross differences occur (Fig. 4b). Except for the contacts on the cell bodies of type XIV, which have a very large standard error, L varies between 213 and 332 nm, which means that the mean surface area varies between 0.05 and 0.12 μm^2. Nevertheless, especially for layers 4 and 5, several tendencies are discernable. For all cell types that have components in both layers (types I, VI, XII, and XIV), the mean size of the contacts in layer 4 is smaller than in layer 5, and for types VI and XII this difference is highly significant ($p<<0.01$). Furthermore, all contacts in layer 5 except those on type XIV, can be considered as a single population ($\bar{L}$ = 308 nm), since no clearly significant differences occur ($p>0.01$, and for most pairs $p>0.05$) as well). The contacts in layer 4, again with the exception of those on type XIV, can also be considered as a single population ($\bar{L}$ = 261 nm). In this way three populations of contacts can be distinguished, viz.: 1) the contacts on type XIV cells, 2) the contacts in layer 5, and 3) the contacts in layer 4. The differences between these populations of contacts are highly significant ($p<<0.01$). It is obvious that the contacts in layer 7 form a fourth population. For the contacts in layers 2 and 3 no general conclusions can be drawn since almost all components present in these layers have synaptic contacts of significantly different sizes. In layer 6 and in layer 1 too few contacts are observed for a statistical interpretation of their size.

The characteristics of the distinct populations of contacts are presented in Figure 5. For simplicity, all contacts in layers 2 and 3 are taken together in this figure. The populations distinguished appear to have characteristic mean values for L and for the diameter,and are all significantly different ($p < 0.01$), except contacts in layer 5 as compared with those in layer 7. It should be noted that in the distribution of

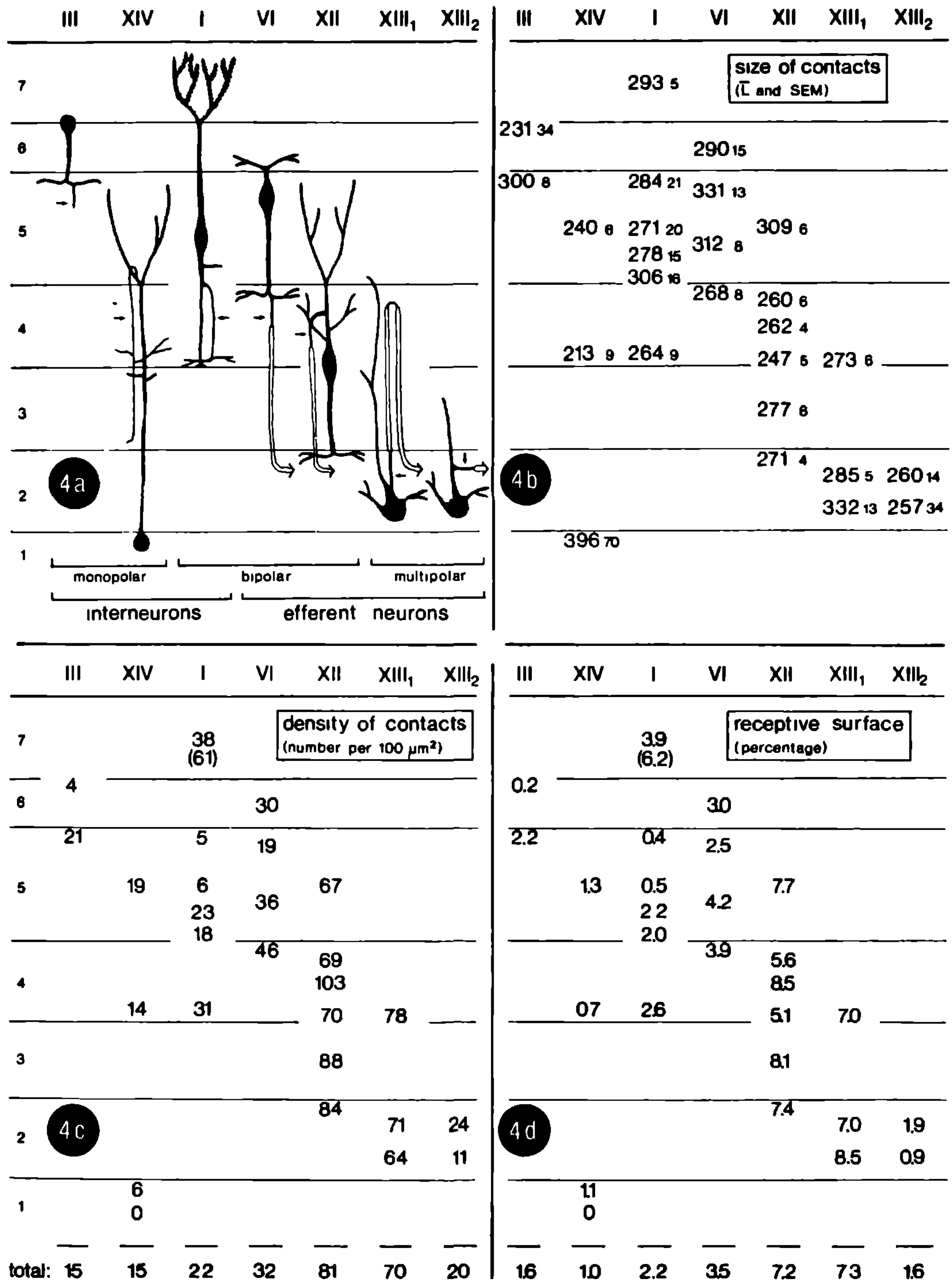
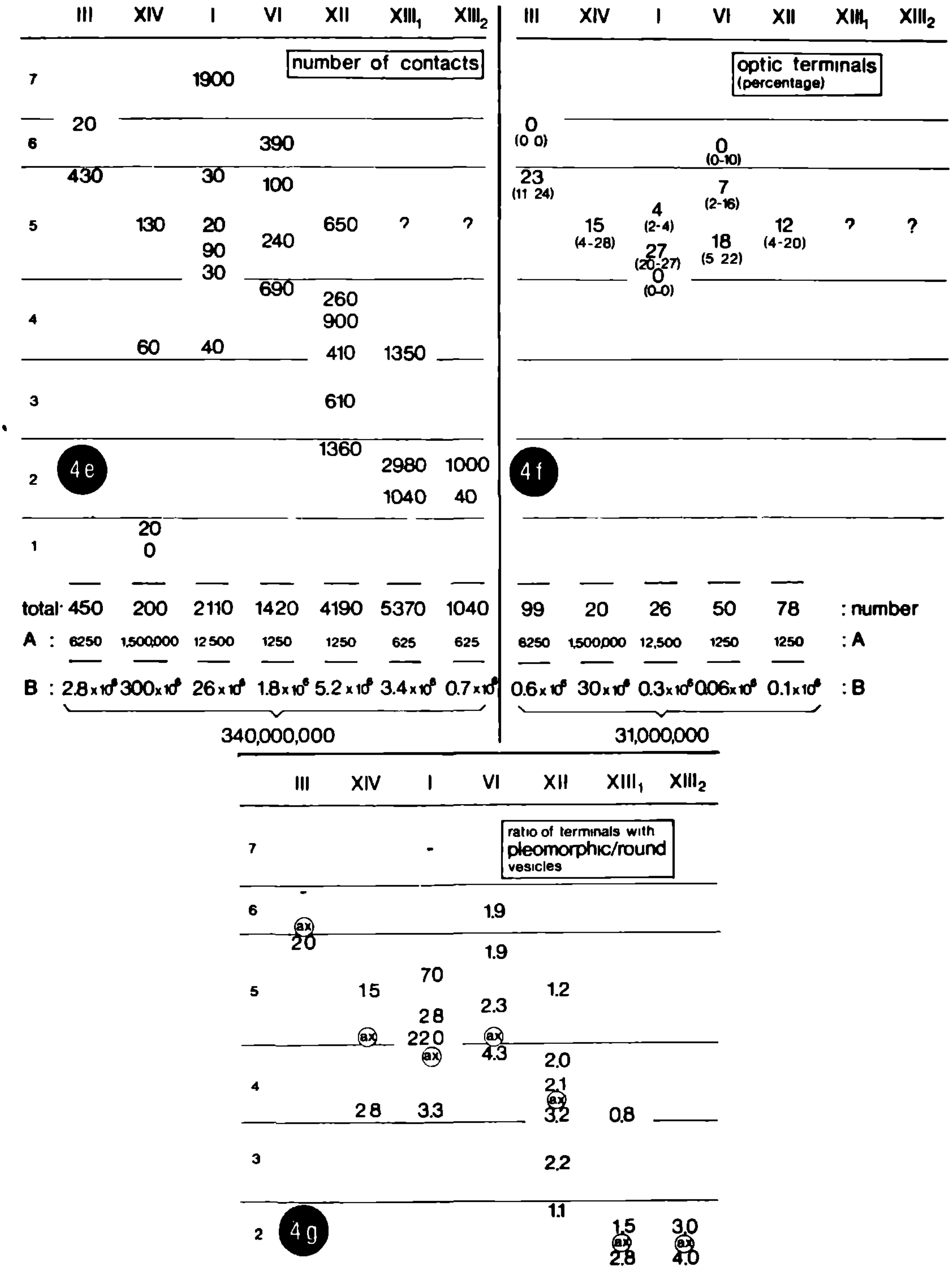


Fig 4 Summary of the quantitative results on the synaptic organization in the goldfish optic tectum The quantitative parameters are presented at their neuronal location The receptive components distinguished are defined in Table 1 and have in this figure the same sequence as in Table 1 4a) Schematic presentation of the cell types investigated and their components distinguished Arrows point to the axons. 4) The size of contacts expressed as L̄ (the mean length of contacts as seen in ultrathin sections) SEM = standard error of the mean 4c) The density of contacts, expressed in number per 100 μm^2 surface area of neuron (calculated with formula 1, see Methods) 4d) The percentage of receptive surface (i e the percentage of neuronal surface showing postsynaptic specialisation) (calculated with formula 2) 4e) The number of contacts (calculated with formula 4) The factor A represents an estimate of the number of cells, derived from Meek



and Schellart ('78) It is the mean of the minimal and maximal estimates given by these authors B is the product of the number of synaptic contacts and the number of cells (A), representing an estimate of the number of contacts of the total population of cells of a certain cell type 4f) The percentage of contacts with optic nerve terminals The main value given represents the most reasonable value (formula 7, see Methods) and the values in parentheses indicate the estimations of the minimal and maximal values respectively (see Methods, formulas 5 and 6, respectively) For A and B, see Figure 4e 4g) The ratio between terminals making contact with the cells studied containing pleomorphic vesicles and those containing round vesicles. Ax Indicates the site of axon origin

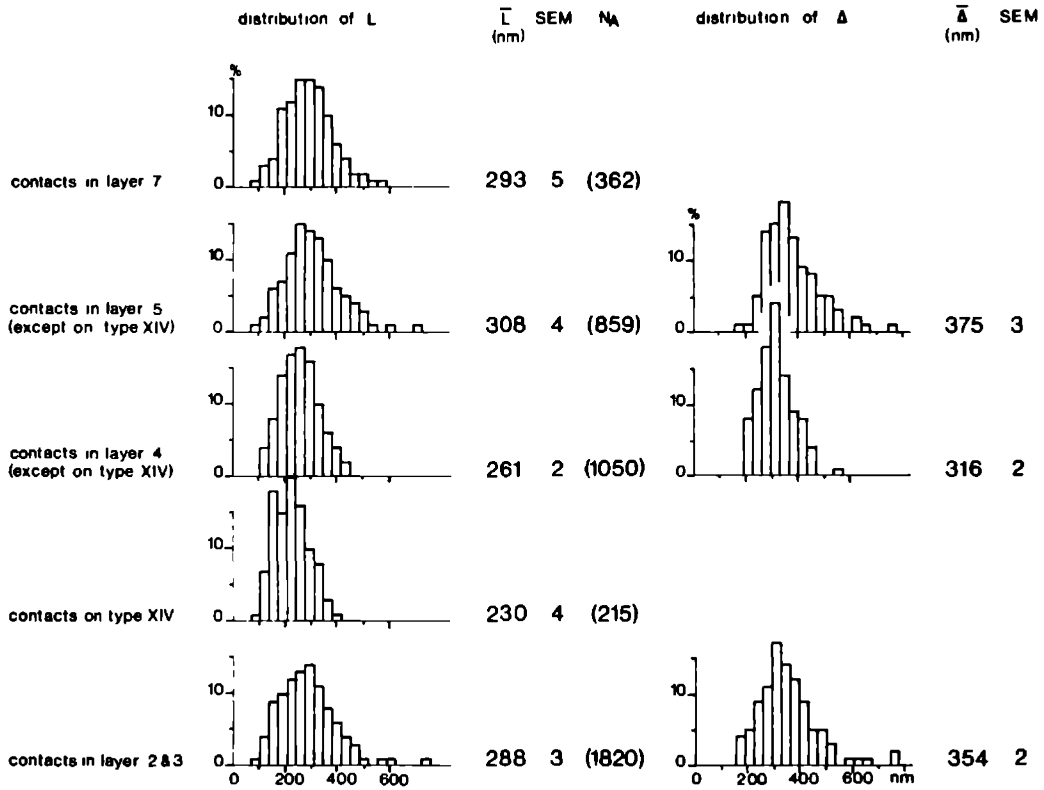


Fig. 5. Characteristics of the different populations of synaptic contacts distinguished on base of their mean size (see Results). The distribution of the diameters was derived from the distribution of L according to the method given by Weibel and Bolender ('73; p. 252). SEM = standard error of the mean

contacts in layers 2 and 3 no subpopulations can be distinguished, in spite of the variability of the size of contacts on the various components in these layers.

The density of synaptic contacts

The results show that all cell types studied have a characteristic averaged density as well as a characteristic density distribution pattern (Table 1; Figs. 4c and 6). The results for the various specimens studied appear largely consistent for each cell type in this respect (Table 1), whereas different type of elements in the same layer, which are totally intermingled in the tectal neuropil, may have very different densities (Fig. 4c). As far as the results appear significant ($p < 0.05$), the following patterns are discernable.

Type III neurons have two regions with a distinct density: the dendrites, with a moderate density of contacts, and a cell body with no or very few synapses. The latter was found not only for the two impregnated examples, but also for several unimpregnated profiles that could be recognized as type III cell bodies by their dilated rough endoplasmatic reticulum (Meek, '81).

Type XIV neurons resemble type III neurons with respect to synaptic density in having dendrites with a moderate density of contacts and a cell body with no or few synaptic contacts. In addition, more superficially located cell bodies differ from more deeply located ones since the former have a few synaptic contacts, whereas the latter have no contacts at all, which was not only observed for the impregnated cells, but also for unimpregnated profiles.

For *type I cells,* three regions can be distinguished on the basis of the density of contacts; 1) the apical dendrites, with a relatively high density; 2) the apical dendritic shaft and the cell body, with a low density; 3) the basal dendrites and the basal dendritic shaft, with an intermediate density.

For *type XII cells*, three regions can be distinguished as well: 1) the apical dendrites, apical dendritic shaft and cell body, with about 70 contacts per 100 μm^2; 2) the dendrites in layer 4 with about 100 contacts per 100 μm^2; and 3) the basal dendritic shaft and basal dendrites with about 85 synapses per 100 μm^2.

Although for *type VI cells* three distinctive regions can be observed as well (Fig. 4c), viz. a relatively low density on the cell body, a relatively high density on the basal dendrites and intermediate values for the dendritic shaft and apical dendrites, this was not consistent for all individual cells studied (Table 1) and consequently not significant for the cell type.

The synaptic properties of the *type* $XIII_1$ *and* $XIII_2$ cells studied differ considerably, in spite of their similar dendritic arborisation pattern. Type $XIII_1$ neurons have a rather high density of contacts on the cell body, the dendrites and on the initial unmyelinated parts of the axons (values found for the latter are 49, 45, 30, and 58 contacts per 100 μm^2). In contrast, the density of synaptic contacts is low for the cell body and dendrites of the type $XIII_2$ cell studied and no contacts at all were observed on the initial part of its axon. Because of the small number of neurons studied per subtype, statistical testing was not possible, in spite of the fact that the total membrane trace investigated for type XIII neurons is substantial (Table 1).

The percentage of receptive surface

Interpretation of this parameter is rather difficult, because both size and density of contacts influence this value (Fig. 4d). Since the mean contact size, however, is rather similar for most components, the percentage of receptive surface appears comparable with the density of contacts. Both values appear to differ in most cases by a factor of about 10 (cf. Fig. 4c–4d). Since the density is expressed as the number per 100 μm^2 surface area, the mean surface area of a contact should be 0.1 μm^2, and consequently the mean diameter 355 nm and $\bar{L}$ 297 nm, which is indeed in agreement with the values found (Figs. 4b and 5). Deviations from this factor 10 reflect deviations in the size of the contacts.

The number of contacts per component of a cell type

The values calculated are presented in Figure 4e, where also the total number per cell type is given. This total number ranges from about 200 (type XIV) to about 5,000 (type $XIII_1$) and is different for all cell types studied. The number of contacts is influenced by the density of contacts as well as by the extension and diameter of the structures upon which they occur (see Fig. 6). For type XIV cells the number of contacts has been calculated for the population of "small" cells (Meek and Schellart, '78). For "large type XIV cells," which are much less numerous than "small" cells, about 300 synaptic contacts may be expected per cell.

When the number of contacts per cell type is multiplied by the estimated number of cells per cell type concerned, an indication of the importance of a certain cell type in tectal circuitry is obtained. The numbers obtained in this way are also given in Figure 4e. The values are calculated for the number of cells given by Meek and Schellart ('78). The population of type XIV cells appears to have by far the most synaptic contacts. The low number of contacts per individual cell is overruled by the large number of cells of this type. Type I is the second type in this respect, having most contacts in layer 7, with fibers from the torus longitudinalis. The series is continued by types XII, $XIII_1$, III, VI, and $XIII_2$, respectively.

The percentage of contacts with optic nerve terminals per component

The results are presented in Figure 4f. Optic terminals, recognized by their pale mitochondria with dilated cristae and their large round vesicles, have exclusively been observed in layer 5. They make contacts with all cell types that have been studied in this layer, but they make only a minor contribution to the total number of synaptic contacts of these cell types. All cell types seem to have a similar percentage of contacts with optic terminals.

The *number* of contacts with optic terminals per cell type is also presented in Figure 4f. Multiplication of these numbers with the estimated number of cells per type reveals that the optic fibers make by far most contacts with type XIV cells, because of the large frequency of occurrence of this cell category. The other types studied receive together only 4% of all optic terminals. For the cell types studied, per tectal half an estimate of about 30 million contacts with optic terminals is obtained (Fig. 4f), which means that optic terminals contribute to only about 10% of the total number of contacts for these types (340 million, Fig. 4e). Since about 200,000 optic fibers are thought to project to the tectum (derived from Schellart, '73), each optic fiber must have about 150 contacts, giving a total number of 30 million contacts.

The ratio between terminals with pleomorphic and round vesicles

It should be mentioned that rather subjective criteria were used to measure this ratio (see Methods) and that, consequently, the results may be used solely for mutual comparison. The ratios found for most cells vary between about one and four, and no characteristic differences between the cell types occur. Only type I cells deviate, since the few presynaptic elements that make contact with their cell bodies and dendritic shafts have almost exclusively pleomorphic vesicles. For all cell types except for type XIV, there is a tendency for the ratio between terminals with pleomorphic and round vesicles to increase as the distance from the axon origin decreases. The high ratio for the presynaptic elements that terminate on the initial unmyelinated parts of the axons of type $XIII_1$ cells (ratio 6.0; $n = 21$) is in agreement with this tendency. The apical dendrites of type I cells were not investigated, since they make contacts exclusively with the marginal axons, originating from the torus longitudinalis (Meek, '81).

Synaptic contacts of identified axons

Apart from the contacts on the distinct receptive components, analyzed in the foregoing paragraphs, the contacts of four groups of presynaptic components were also studied, viz. the axons of type I and type XIV cells, both recognized by means of Golgi impregnation (see Fig. 1), and optic and toral fibers (marginal axons), identified by their distinct ultrastructural characteristics or their characteristic location respectively (see Meek, '81). The size of the contacts made by these axons is given in Table 2. Type XIV axons have significantly smaller contacts than the axons of type I, and the axons of both types of interneurons have significantly smaller contact zones than the afferents from the retina and from the torus longitudinalis ($p < 0.01$).

The density and number of contacts made by the optic and toral afferents could not be calculated with the formulae given in the Methods section, since no a-select sample of their membrane was measured, but only those parts that made synaptic contacts. An estimation of the number of contacts made by optic fibers, however, could be obtained in an indirect way, as presented in the paragraph on the percentage of contacts with optic nerve terminals. The density and number of contacts calculated for the individual axons of type I and type XIV cells by means of formulas (1) and (3) (see Methods) is given in Table 2 as well. The sample is too small for general conclusions, and gives only an impression of the order of magnitude. With respect to the total number of contacts per individual axon, comparison of Figure 1 of the present paper with Figures 3 and 16 of Meek and Schellart ('78) shows that for axons of types I and XIV up to 500 synaptic contacts may be expected, since the length of the collaterals analyzed in the present study is substantially smaller than the total length of axon collaterals observed for several examples of these neurons.

DISCUSSION

Comparison of the present results with literature reveals that the mean size of synaptic contacts in the goldfish tectum (Fig. 5) is quite comparable with the average size of contacts in normal trout tectum ($\overline{L} = 260$ nm; Jesserich and Rahmann, '79), especially when one considers the large influence of contacts of the numerous type XIV cells on the overall measurements as given for the trout tectum. The sizes of tectal contacts are also similar to those of contacts in the rabbit superior colliculus ($\overline{L} = 257$ nm; Vrensen and de Groot, '77). In contrast, contacts in the cerebral cortex appear to be larger (Jones et al., '74; guinea pig; Vrensen et al., '80, rabbit).

It should be mentioned that rather large differences exist between the mean size of tectal contacts measured on identified dendrites and cell bodies (Fig. 5) and those of identified axon terminals (Table 2), the former being substantially larger than the latter. Consequently, these axons may constitute only a portion of the total number of synapses present on the dendrites and cell bodies measured, and groups of axons establishing larger contacts

Table 2. The size, density and number of synaptic contacts of identified axons in the goldfish tectum

Type of presynaptic element	Position (layer)	$L \pm SEM$ (N_A)			Density (per axon)		Number (per axon)	
Toral fibers	7	293	5	(362)				
Optic nerve fibers	5	272	13	(43)				
Axons of type I cells	3/4	237	7	(91)	23	53	25	75
Axons of type XIV cells	4	188	6	(80)	2	33	5	80

have to account for the remaining synapses. The axons of several types of uninvestigated afferents and interneurons are possible candidates (Meek and Schellart, '78). It is, however, also possible that a portion of the heterogeneous population of type XIV neurons has axons with larger contacts than the specimens investigated up to now.

To our knowledge, the only quantitative results for the density of synaptic contacts on identified neurons in the central nervous system are those published by Davis and Sterling ('79). Using reconstruction of serial sections, these authors found values for cell bodies in the rat visual cortex ranging from 8 to 47 contacts per 100 μm^2 surface, values similar to those found in the present study for tectal neurons.

The spiny dendrites of type I neurons appear to have the same or even a lower density of contacts than several nonspiny dendrites (Table 1, Fig. 6), even when the surface enlargement by the spines is neglected (Table 1, values in parentheses). This confirms the idea that the function of spines is not simply that of surface enlargement, but may be a more specific electrophysiological one (Peters et al., '76). The high density of contacts on several nonspiny dendrites and the fact that several synaptic contacts between the spines (about 10%; see also Meek, '81) also occur on the spiny dendrites of type I neurons indicate that the counting of spines does not necessarily give a good impression of the numbers of synaptic contacts.

The values calculated for the number of contacts per neuron (Fig. 6) vary from about 200 (type XIV) to about 5000 (type $XIII_1$). Compared with values calculated for the cerebral cortex of several mammals (values ranging from 5,000 to 60,000 synapses per neuron; Cragg, '74, monkey and human; Vrensen, '78, rabbit), this is rather low.

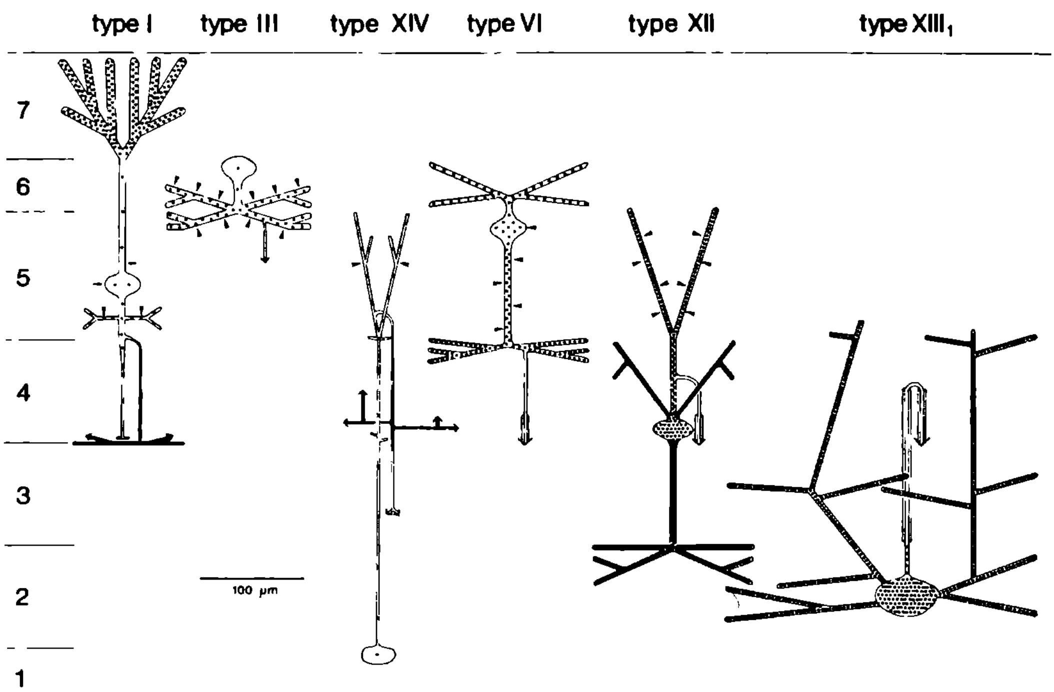


Fig. 6. Schematic visualization of the densities and numbers of contacts calculated for the tectal cell types investigated (except for type $XIII_2$ because only one cell of this type was studied). Each dot represents ten synaptic contacts. White structures (with black dots) are postsynaptic elements and black structures (with white dots) are presynaptic elements. Each arrowhead indicates ten contacts with optic nerve terminals. Each small arrow (type I) indicates a single contact with an optic nerve terminal. The horizontal dendritic extension and the total length of the dendrites are drawn to scale according to Meek and Schellart ('78), partly based on a reinvestigation of their material ($\overline{M}_{type}$, see Methods). The diameters of the structures drawn indicate their mean circumference. The length of structures ending with arrows is uncertain. The numbers 1–7 indicate the tectal layers.

Optic nerve terminals contribute to about 10% of all tectal synaptic contacts in goldfish (Figs 4e, 4f, and 6), and appear to occur on all cell types with postsynaptic elements in layer 5. Therefore, optic input appears not to be confined to a certain cell type but is more generally distributed over all dendritic and somatic structures in layer 5. A number of 150 contacts per optic nerve fiber was calculated (see Results), which means that optic fibers must have long and/or branching terminal parts. This arrangement has been only occasionally reported for the teleostean tectum (Ramon Cajal, 1899; Potter, '76), but is commonly observed in the tectum of other vertebrates (e.g. Ramon Cajal, 1898; Lazar and Szekely, '67; Stone and Freeman, '71; Potter, '76).

The frequency of occurrence of optic nerve terminals estimated in the present study is in substantial agreement with the numbers recently published in two other reports on the teleostean tectum. Airhart and Kriebel ('80) found for goldfish that the retinal terminal population makes up 18% of the total terminal population in the *superficial gray and plexiform layer* (layer 5), an average value resembling the values calculated per cell type in the present study (Fig. 4f). Ito et al. ('80) reported for *Holocentrus rufus* that 16% of the terminals in the *superficial gray and plexiform layer* is of the S_2 type and consequently comes from the retina. However, comparison with all tectal synaptic contacts (not only those in layer 5) yields for *H. rufus* a much smaller percentage (less than 5%, see Table 1 of Ito et al , '80) than for goldfish (about 10%, present results). This is rather unexpected, since *H rufus* is visually more developed than the goldfish (Schroeder et al , '80).

In spite of the complexity of the neuronal organization of the tectum, some specific conclusions regarding its synaptic organization can be drawn. One of the main findings is the observation that homologous dendrites and cell bodies have a comparable density of contacts (Table 1), whereas different components in the same layer may have significantly different synaptic densities (Figs. 4c and 6) This strongly suggests that the density of contacts is primarily determined by the postsynaptic elements of the tectal cells, and is not randomly distributed; nor does it depend primarily on the type of presynaptic element involved in the synaptic contacts (in the latter case one should have expected a relation with the tectal lamination pattern, as, e.g., found for the size of the contacts) Within certain limits, each tectal cell type apparently has a precoded density distribution along its various receptive components. This confirms the more generally formulated idea of Cragg ('74) that "it is the number of dendritic sites that will accept a synapse that limits the connectivity of the nervous system," and agrees with findings that in developing nervous tissue the postsynaptic membranous specializations occur earlier than the facing presynaptic dense projections (see e.g. Pfenninger and Rees, '76, Muller et al , '80). The findings of a characteristic pattern of synaptic distribution on each tectal cell type (Fig. 6) are also in agreement with the idea that the morphological differences between the various cell types distinguished by Meek and Schellart ('78) reflect functional differences. These tectal cell types not only have characteristic locations and extensions of their dendrites, indicative of the kind of input the cell type receives and their receptive field size (see Meek and Schellart, '78), but their characteristic pattern of densities of contacts indicates, in addition, a mechanism regulating the intensity of different types of input.

It should be mentioned that the density distribution is not only characteristic of each cell type but can also to a certain extent be related to a distinction between mono-, bi-, and multipolar cells (see Figs 4 and 6). Monopolar cells (types III and XIV) have two distinct regions: a cell body, with very few or no synaptic contacts, and a dendritic shaft and dendrites with a moderate density of contacts (about 20 contacts per 100 μm^2 surface area). Bipolar cells (types I, VI, and XII) tend to have a complex synaptic pattern with several distinct regions, whereas multipolar cells (types $XIII_1$ and $XIII_2$) have comparable densities on all parts of their receptive surface, the initial part of the axon included. No basic differences seem to exist between the surface membranes of dendrites, dendritic shafts, and cell bodies, since each of these types of postsynaptic components has a comparable variety of synaptic densities (Fig. 6)

With respect to the size of the tectal contacts, the main observation is that most postsynaptic components in a particular layer, especially in layers 4 and 5, have contacts of a similar, characteristic size (Figs. 4b and 5). This might suggest that these components are contacted by the same population of presynaptic elements and that the size of synaptic contacts, in contrast to their density, is primarily determined by the presynaptic element. As each layer contains its own, characteristic population of axons (Meek and Schellart, '78) and consequently its own population of presynaptic elements, these can be held responsible for the characteristic size of contacts per layer. This suggestion is supported by the observation that identified types of axons indeed have a characteristic mean size of contacts (Table 2). The smaller size of the contacts in layer 4 compared with those in layer 5 as well as in layers 2 and 3 (Fig. 5) can be related to the absence of afferents in layer 4 (Vanegas, '75; Meek, '81) and suggests that tectal afferents on average establish larger contacts than axons of tectal interneurons, which is confirmed by the observations on the four distinct populations of axons studied (Table 2). The fact that the dendrites of type XIV cells have smaller contacts than the other structures in the same layer could indicate that they are contacted by only a subpopulation of the presynaptic elements present. As regards layers 2 and 3, our observations do not allow any specific conclusion.

The present study has shown that the combination of Golgi-EM and quantitative stereological methods can provide interesting information about the synaptic organization of a brain center, provided that the cell types are well defined. The measurements to be performed are simple, involving only measurements of lengths and counting of structures; the special stereological formulae are derived from those generally applied to other subjects, and are easily understood. A serious drawback of the method is, however, that it is rather laborious and time-consuming.

One of the main purposes of neuroanatomical studies is to obtain precise knowledge of the afferent and efferent connectivity patterns of the cell types present in a brain center. For this purpose combined light and electron microscopy has to be applied since the light microscope is the most appropriate instrument for the identification of neurons, whereas the synaptic contacts between neurons can be visualized only in the electron microscope. Combination of Golgi impregnation with electron microscopy indeed enables identification of one side of a synaptic contact (e.g. Le Vay, '73; Blackstad, '75; Fairén et al., '77; Somogyi, '78; Meek, '81) and by combination of Golgi-EM with anterograde degeneration (Blackstad, '75; Somogyi, '78; White, '78; Peters et al., '79) or with retrograde HRP transport (Somogyi et al., '79), both sides of a synaptic contact can be identified. However, for an evaluation of the significance of such identified connections, data on their density and number are required. The quantitative analysis of Golgi-EM sections applied in the present study offers a promising way to obtain such data. By combination of this type of analysis with neuroanatomical tracing techniques (degeneration, HRP, autoradiography of labelled amino acids), it will be possible to study neuronal circuitry in great detail.

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FUNCTIONAL ORGANIZATION OF THE TECTUM MESENCEPHALI OF THE GOLDFISH

Introductory notes

In chapters II, III and IV of the present thesis the morphological characteristics of the tectal cell types and the synaptic properties of six of these cell types have been described qualitatively and quantitatively. In the following discussion our findings will be combined with data from literature in order to analyse the functional implications of tectal cytoarchitecture and synaptology. For this purpose, at first the present knowledge concerning tectal afferents will be summarized. Secondly, an attempt will be made to indicate the function of the various cell types in tectal circuitry on guide of the fairly strict lamination pattern of the tectal afferents as well as of the dendrites and axons of the tectal neurons, and on basis of quantitative considerations concerning the number and distribution of synaptic contacts. The tentative conclusions will be compared with available electrophysiological data. The last part of the discussion summarizes the present knowledge concerning tectal efferents.

1 TECTAL AFFERENTS

1.1 Retinal efferents

The best-known source of tectal afferents is the retina, not only in teleosts, but in all vertebrates. In goldfish, the termination pattern of retinal efferents or optic fibers has been analysed experimentally by Sharma ('72) with the degeneration technique and by Springer and Landreth ('77) with the autoradiographic tracing method. Their findings, complemented with some results of Finger and Karten ('78) and Grover and Sharma ('81), are summarized in figure V.1. The position and size of the various retinal targets in the goldfish is shown in figures V.3, V.4 and V.5. Apart from the massive contralateral tectal projection, which is topographically organized (see the General Introduction), retinal efferents appear to terminate in several contra- as well as ipsilateral diencephalic nuclei (fig. V.1). These include contralaterally the area preoptica and nucleus preopticus in the hypothalamus; the area ventrolateralis and the nucleus dorsolateralis in the thalamus, and four pretectal nuclei, viz. the area pretectalis, the nucleus pretectalis, the nucleus geniculatus lateralis and the nucleus corticalis. Ipsilateral projection regions include the nucleus preopticus, nucleus dorsolateralis thalami, area pretectalis and nucleus pretectalis.

Contralateral retinal projections have also been analysed in a large number of other teleosts (Ebbesson, '68; Campbell and Ebbesson, '69; Vanegas

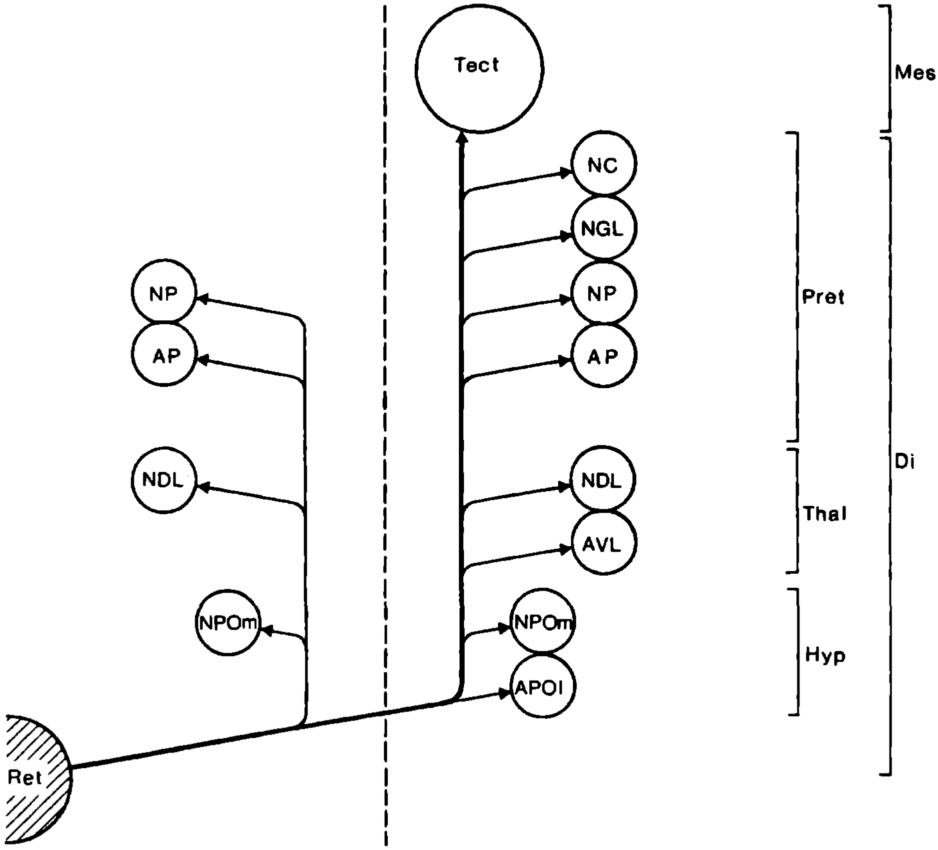


Fig. V.1. Schematic representation of present knowlegde concerning retinal efferents in the goldfish. For references, see text, section 1.1. For abbreviations: see pag. 110.

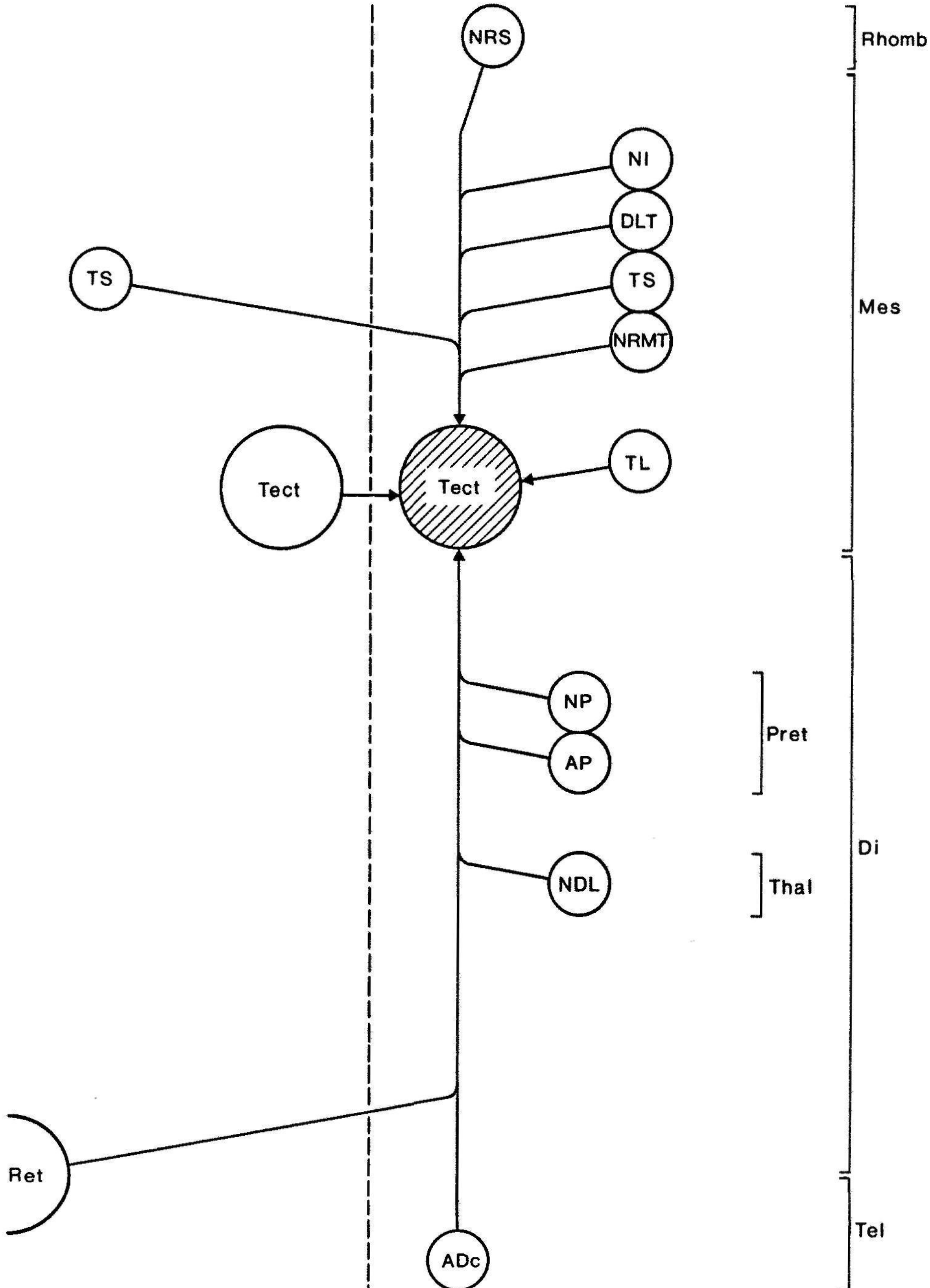


Fig. V.2. Schematic representation of present knowledge concerning tectal afferents in the goldfish. For references ,see text, section 1.2. For abbreviations see pag. 110.

ADc	area dorsalis centralis
AP	area pretectalis
APOl	area preoptica lateralis
AVL	area ventrolateralis thalami
ChO	chiasma opticum
Cereb	cerebellum
Di	diencephalon
DLT	nucleus dorsalis lateralis tegmenti
FRl	formatio reticularis lateralis
FRm	formatio reticularis medialis
Hyp	hypothalamus
IPL	inner plexiform layer
Mes	mesencephalon
NC	nucleus corticalis
NDL	nucleus dorsolateralis thalami
NGL	nucleus geniculatus lateralis
NI	nucleus isthmi
NO	nervus opticus
NP	nucleus pretectalis
NPOm	nucleus preopticus, pars magnocellularis
NR	nucleus rotundus
NRMT	nucleus rostralis mesencephali tegmenti
NRS	nucleus reticularis superior
Pret	pretectum
Ret	retina
Rhomb	rhombencephalon
SAC	stratum album centrale
SFGS	stratum fibrosum et griseum superficiale
SGC	stratum griseum centrale
SM	stratum marginale
SO	stratum opticum
SPV	stratum periventriculare
Tect	tectum mesencephali
Tel	telencephalon
Thal	thalamus
TL	torus longitudinalis
TrO	tractus opticus
TrOd	tractus opticus dorsalis
TrOv	tractus opticus ventralis
TS	torus semicircularis
TTB	tractus tecto bulbaris

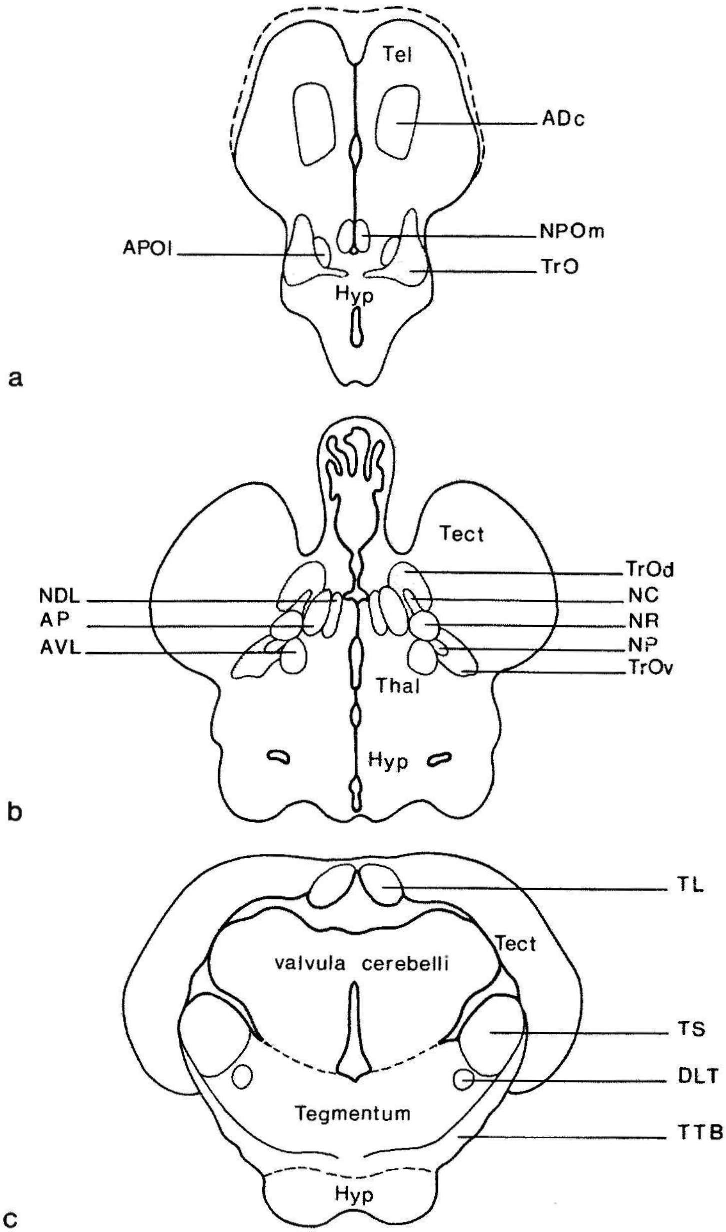


Fig. V.3. Transverse sections through the brain of the goldfish, indicating a number of nuclei connected with the tectum. The drawings are based upon the atlas of Peter and Gill ('75) and own observations. a: through the tractus opticus; b: through the rostral tectum; c: through the caudal tectum. The levels of sectioning are indicated in fig. V.4. For abbreviations: see pag. 110.

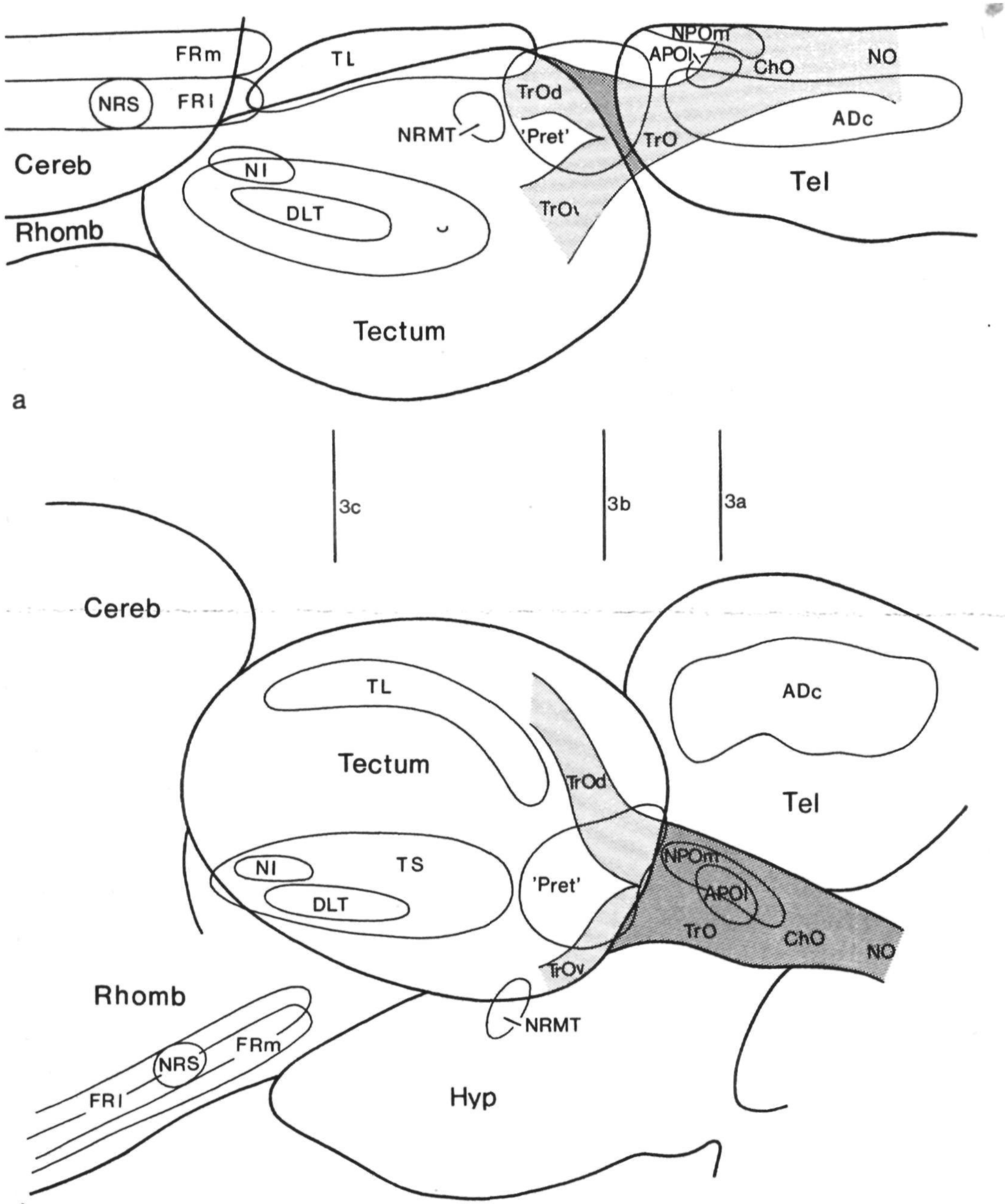


Fig. V.4. Topographical projections on a horizontal (a) and sagittal (b) plane of part of the right half of the brain of the goldfish, indicating the position and size of nuclei connected with the tectum. The projections of forebrain nuclei are based on the atlas of Peter and Gill ('75), the projections of the remaining nuclei on the drawings of Grover and Sharma ('79 and '81) and on own observations. 3a, 3b and 3c indicate the sections drawn in fig. V.3. For abbreviations:see pag. 110. The region "Pret" is presented in fig.V.5.

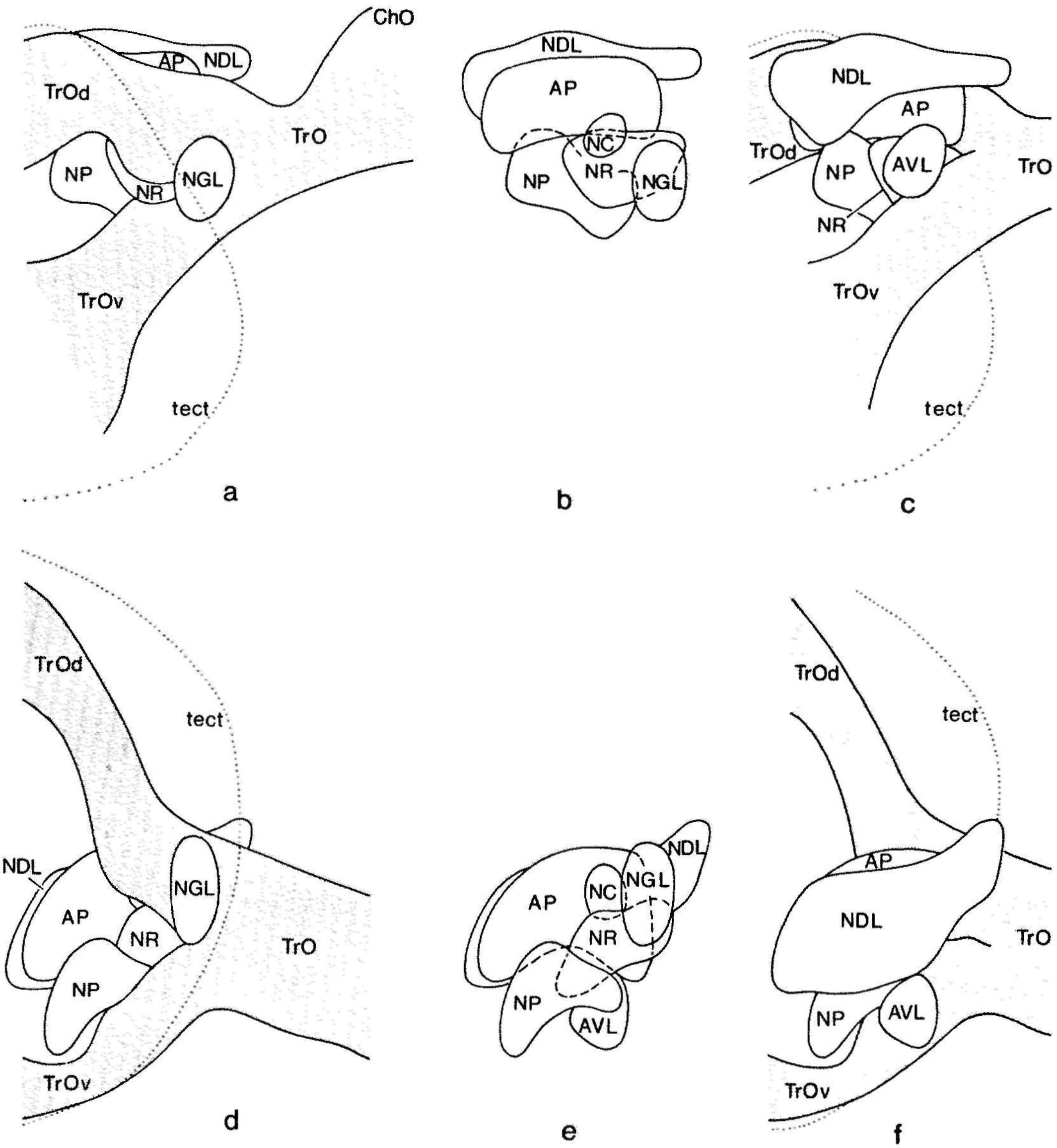


Fig. V.5. Topographical projections on a horizontal (a,b,c) and sagittal (d,e,f) plane of the pretectal and dorsolateral thalamic region, indicating the position and size of some nuclei connected with the tectum. The projections are based on the atlas of Peter and Gill ('75). a: dorsal view; b: dorsal view without tractus opticus; c: ventral view; d: lateral view; e: lateral view without tractus opticus; f:medial view.For abbreviations: see page 110.

and Ebbesson, '73; Anders and Hibbard, '74; Gulley et al., '75; Repérant and Lemire, '76; Repérant et al., '76; Voneida and Sligar, '76; Peyrichoux et al., '77; Finger and Karten, '78; Pinganaud and Clairambault, '79; Meyer and Ebbesson, '81). Several differences between the various studies can be noticed with respect to the thalamic-pretectal projection. For instance, the nucleus rotundus is a retinal target in the goldfish according to Sharma ('72). However, this was not confirmed by Finger and Karten ('78) and was equally not found in other teleosts. In the latter, however, optic fibers appear to terminate frequently in the contralateral nucleus pretectalis which was not reported by Sharma ('72) for goldfish, but later still could be shown in this species with autoradiographic methods (Finger and Karten, '78; Grover and Sharma, '81).

A detailed discussion on all differences between the various studies concerning retinal targets is beyond the scope of this section, but it should be noticed that not only technical and species differences may be involved, but also factors such as the large variety in size and position of the pretectal nuclei in the different teleosts studied, combined with the lack of well-established criteria to recognize homologous nuclei in this region and the use of different nomenclatures by the various authors (see e.g. Schnitzlein, '62; Peter and Gill, '75). Nevertheless, the basic pattern of contralateral optic projections seems to be the same for all teleosts and consists of terminations in: 1) the preoptic hypothalamic region; 2) nuclei in the dorsolateral and ventrolateral thalamus; 3) pretectal nuclei and 4) the tectum mesencephali.

Some authors mention a fifth region of termination of optic fibers, viz. the nucleus opticus accessorius (Campbell and Ebbesson, '69; Anders and Hibbard, '74; Repérant et al., '76; Peyrichoux et al., '77), which lies just in the rostral tegmentum mesencephali according to Repérant et al. ('76) and Peyrichoux et al. ('77). Because of the lack of good criteria for comparison and of a consistent nomenclature, it might be possible that this nucleus opticus accessorius is homologous to the area ventrolateralis thalami of Sharma ('72; see figs. V.3 and V.5), the nucleus opticus ventromedialis of Vanegas and Ebbesson ('73) or the nucleus opticus basalis of Voneida and Sligar ('76) and Pinganaud et al. ('79), which are considered as diencephalic centers. The names nucleus opticus accessorius or nucleus opticus basalis seem to be misleading, since this structure might not be homologous to the accessory optic system in other vertebrates because it lacks connections with the cerebellum and oculomotor nuclei in both goldfish and carp (Finger and Karten, '78; Grover and Sharma, '81; Luiten, '81).

The ipsilateral retinal projections in the goldfish (fig. V.1) have only been shown by means of autoradiography (Springer and Landreth, '77) and similar projections have only occasionally been described for other teleosts. Voneida and Sligar ('76) reported ipsilateral retinal projections in *Astyanax mexicanus* and Repérant et al. ('76) have shown ipsilateral projections in a number of other teleosts. The latter authors suggest that all teleosts have some ipsilaterally terminating retinal fibers, which, however, would only be demonstrable with special and sensitive autoradiographic methods.

For an adequate evaluation of the retinal projections, quantitative aspects are important as well. Both degeneration and autoradiographic experiments clearly demonstrate that the ipsilateral projections are much less dense than the contralateral projections and that the bulk of retinal efferents projects to the tectum mesencephali. Especially the small size of the diencephalic retinal targets compared with the mesencephalic tectum should be realized (see figs. V.3, V.4 and V.5). To give some impression: the total volume of the goldfish tectum is about 4500 mm^3, whereas the total volume of the pretectal and thalamic retinal targets in the goldfish is not more than 200 mm^3. The nucleus rotundus, the most easily distinguishable pretectal nucleus, contains about 500 neurons, (derived from cell counts in Nissl section), and the nucleus corticalis, the smallest pretectal nucleus in the goldfish, consists of only 6 to 8 neurons (Schnitzlein, '62). These numbers are very small compared with the total number of tectal neurons (chapter III).

1.2 Non-retinal tectal afferents

Tectal afferents have recently been studied in the goldfish by Grover and Sharma ('81) with the aid of retrograde HRP transport. Their results are summarized in figure V.2 and the nuclei mentioned in figure V.2 are indicated in figures V.3, V.4 and V.5. Luiten ('81) has recently published similar results for the tectum of the carp.

Apart from the retina, one telencephalic area (area dorsalis centralis), three diencephalic nuclei (nucleus dorsolateralis, area pretectalis and nucleus pretectalis), six mesencephalic structures (torus longitudinalis, contralateral tectal half, nucleus of the rostral mesencephalic tegmentum, torus semicircularis, nucleus dorsolateralis tegmenti and nucleus isthmi) and one rhombencephalic nucleus (nucleus reticularis superior) project to the goldfish tectum (fig. V.2). The projection of the telencephalic area dorsalis centralis has also been described for *Eugerres plumieri* and *Holocentrus* (Vanegas and Ebbesson, '76) and the carp (Ito and Kishida, '77; Luiten, '81). The three diencephalic nuclei that project to the tectum all receive retinal input (fig. V.1). In addition, Luiten ('81) has described for the carp that also a hypothalamic retinal target, viz. the nucleus preopticus, projects to the tectum, and that in this teleost not only ipsi-, but also contralateral projections from pretectum to tectum occur. The pretectal nuclei project in goldfish to the rostral tectum (Grover and Sharma, '81).

Apart from the retinotectal fibers, three other projections show a certain degree of topographical organization, viz. the afferents from the contralateral tectum, from the torus longitudinalis and from the nucleus isthmi.

The commissural intertectal fibers, shown in several teleosts (*Eugerres plumieri* and *Holocentrus*: Ebbesson and Vanegas, '76; *Astyanax hubbsi*: Sligar and Voneida, '76; goldfish: Grover and Sharma, '79, '81; and carp: Luiten, '81) preferentially connect homotopic tectal regions (Ebbesson and Vanegas, '76; Grover and Sharma, '79, '81). The toro-tectal projection, extensively studied by Ito and Kishida ('78) in the carp, appears to have a rostro-caudal topographical organization. Afferents to the torus longitudinalis originate almost exclusively in the valvula cerebelli (Ito and Kishida, '78), a cerebellar structure only present in actinopterygians, just as the torus longitudinalis itself (see Nieuwenhuys, '81). The isthmo-tectal projection has recently been studied extensively in the teleost *Navodon modestus* by Sakamoto et al. ('81) and Ito et al. ('81b) and appears to have a detailed topographical organization. Afferents to the nucleus isthmi originate in the ipsilateral tectum mesencephali as well as in the ipsilateral nucleus pretectalis (Ito et al., '81a).

The remaining tectal afferents originate in three mesencephalic tegmental nuclei and in the rhombencephalic reticular formation (fig. V.2). The nucleus of the rostral mesencephalic tegmentum (NRMT) of Grover and Sharma ('81) is called nucleus ruber by Luiten ('81) but does not receive a projection from the cerebellum (Grover and Sharma, '81), which renders the latter name disputable (Grover and Sharma, '81). The torus semicircularis is the largest nucleus of the mesencephalic tegmentum (figs. V.3 and V.4) and receives most of its input from auditory- and lateral line sensory systems (Ito, '74; Knudsen, '77; Nieuwenhuys, '81). The torus semicircularis has in the goldfish, apart from the ipsilateral tectal projection, a small contralateral projection to the tectum (Grover and Sharma, '81), whereas in the carp the neighbouring dorsolateral tegmental nucleus (or nucleus profundus mesencephali) has a contralateral projection to the tectum (Luiten, '81). The most caudal structure that projects to the tectum is the rhombencephalic nucleus reticularis superior, both in the goldfish and the carp. Tectal afferents from the torus semicircularis, nucleus dorsolateralis as well as the reticular formation preferentially terminate in the caudal part of the tectum (Grover and Sharma, '81).

1.3 The lamination pattern of tectal afferents

The present knowledge concerning the laminar distribution of afferent terminals in the goldfish tectum is summarized in figure V.6. The most detailed results have been obtained by means of anterograde tracing techniques, which clearly visualize afferent termination patterns. Additional information, although less precise, has been inferred from retrograde labeling experiments in which HRP was injected in the tectum at different depths.

The laminar distribution of retino-tectal terminals has been analysed in the goldfish by Sharma ('72) with the degeneration technique, by Neale et al.

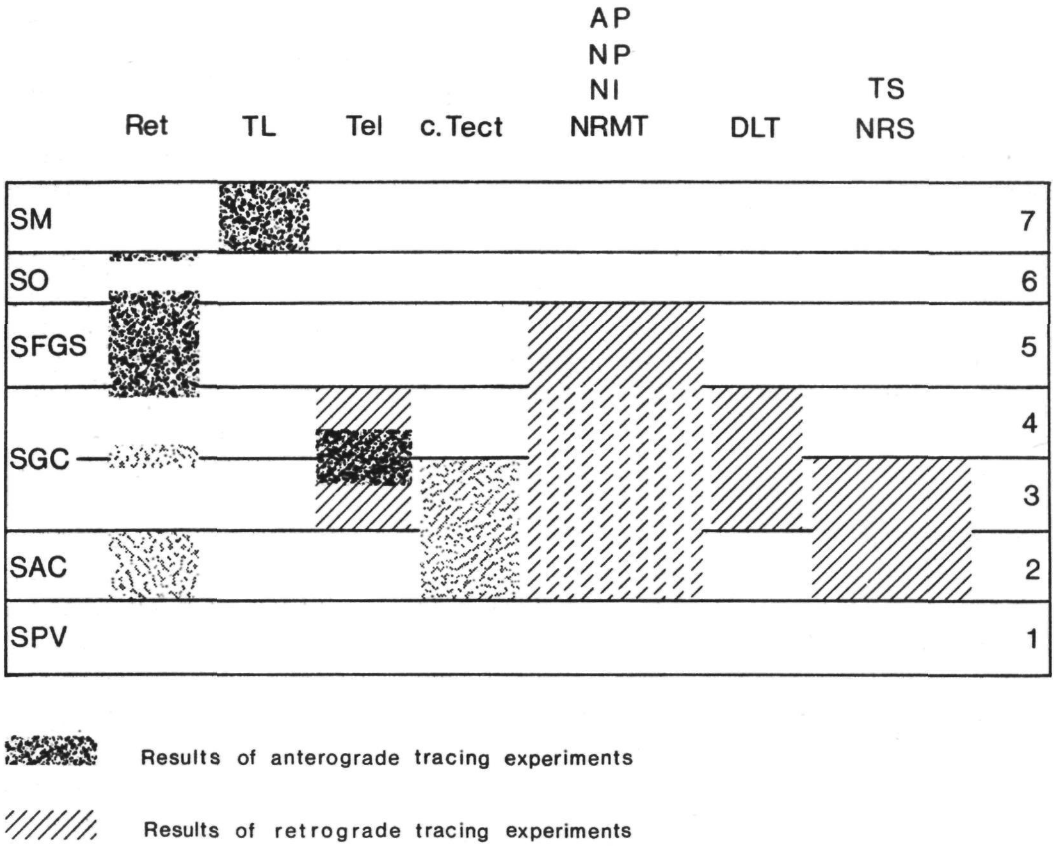


Fig. V.6. Summarizing scheme of the lamination pattern of tectal afferents in the goldfish. For references: see text, section 1.3. For abbreviations: see pag. 110. The interrupted hatching in the fifth column is meant to indicate that the experiments performed do not allow for conclusions about terminals in this zone.

('72) and Landreth et al. ('75) with autoradiography and by Schmidt ('79) with the cobaltchloride technique. These studies have revealed optic fiber terminationsthroughout layer 5, in the superficial part of layer 6 and, especially in the rostral tectum, a sparse optic projection to layer 2. A similar pattern has been described for many other teleosts (Ebbesson, '69; Campbell and Ebbesson, '69; Vanegas and Ebbesson, '73; Anders and Hibbard, '74; Landreth et al., '75; Gulley et al., '75; Voneida and Sligar, '76; Repérant and Lemire, '76; Repérant et al., '76; Peyrichoux et al., '77; Luckenbill-Edds and Sharma, '77; Pinganaud and Clairambault, '79; Meyer and Ebbesson, '81). In addition, autoradiographic and cobalt tracing have revealed a sparse projection to layer 3/4 in the goldfish (Neale et al., '72; Landreth et al., '75; Schmidt, '79), which is also described for some other teleosts (Repérant and Lemire, '76; Repérant et al., '76; Peyrichoux et al., '77; Meyer and Ebbesson, '81). Since such a projection has never been found with degeneration techniques, it has been suggested that it might be arteficial, due to transneuronal transport of aminoacids via type I neurons (Vanegas et al., '81). However, anterograde HRP-tracing and cobalt tracing revealed such a projection as well (Peyrichoux et al., '77; Schmidt, '79), and it is doubtful whether such substances are transported transneuronally.

Afferents from the torus longitudinalis terminate in one single tectal layer, viz. the superficial marginal layer (layer 7). This layer is only present in actinopterygians, as is the torus longitudinalis. Although the toral origin of the unmyelinated marginal fibers has only been demonstrated experimentally in the carp (Ito and Kishida, '78), the typical characteristics of the marginal axons in different teleosts (Ito, '70; Laufer and Vanegas, '74; Ito et al., '80; present thesis) as well as the close correlation between the degree of development of the torus and the marginal layer in a large number of teleosts (Kishida, '79) indicate that the torus projects to the same tectal layer in all teleosts, including goldfish.

The reports about the telencephalic tectal afferents are somewhat confusing. The degeneration experiments of Vanegas and Ebbesson ('76) on *Eugerres* and *Holocentrus* clearly show termination of telencephalic efferents in the ipsilateral stratum griseum centrale, especially in layer 3/4, with some additional termination in layers 6 and 2 in *Holocentrus*. This distribution was confirmed in an EM-study (Ito et al., '80). In contrast, Marotte and Mark ('75) and Airhart ('79) have reported for the crucian carp and the goldfish, respectively,ipsi- as well as contralateral degeneration throughout layers 3, 4 and 5 after telencephalic lesions. However, after tectal HRP injections both in goldfish and carp only ipsilateral telencephalic cells were labelled, and this could only be achieved when the injection site included the stratum griseum centrale (layers 3 and 4) of the tectum (Ito and Kishida, '77; Grover and Sharma, '81; Luiten, '81). This means that the results of Marotte and Mark ('75) and Airhart ('79) are most likely due to aspecific degeneration or other aspecific reactions, which may occur under certain experimental conditions (see e.g. Marotte, '81). Consequently, in all teleosts investigated the telencephalic fibers seem to terminate for the major part in the ipsilateral SGC, with a special concentration in layer 3/4 (Vanegas and Ebbesson, '76; Ito and Kishida, '77; Grover and Sharma, '81; Luiten, '81).

The layers of termination of intertectal fibers have only been determined clearly in the teleosts *Eugerres* and *Holocentrus* with the aid of light- and electron microscopical degeneration studies (Ebbesson and Vanegas, '76; Ito, et al., '80). In these species the intertectal fibers terminate predominantly in layer 3, and for a small portion in layer 2. In three other reports (Sligar and Voneida, '76: dealing with *Astyanax hubbsi*; Grover and Sharma, '79: goldfish; Luiten, '81: carp) the termination level of intertectal fibers was not indicated explicitly, but judging from the drawings published in these papers, in these teleost intertectal fibers also terminate in deep tectal layers (layers 2 and 3). The massive degeneration found in layer 7 after section of the intertectal commissure by Marotte and Mark ('75) is probably due to damage of the torus longitudinalis (cf. Ito and Kishida, '78).

The lamination pattern of the remaining tectal afferents can only be inferred from a study by Grover and Sharma ('81), who placed HRP injections at different levels in the goldfish tectum. They conclude that efferents from the area pretectalis, nucleus pretectalis, nucleus of the rostral mesencephalic tegmentum and nucleus isthmi terminate predominantly in the SFGS (layer 5), and that the nucleus dorsolateralis thalami projects to the mid-tectal level (SGC). Terminals from the torus semicircularis and nucleus reticularis superior could only be labeled with deep tectal HRP-injections, including the SAC (layer 2). It has already been mentioned that these results are less conclusive that those based on degeneration or autoradiographic experiments. Apart from the difficulty of the rather large size of HRP injections compared with the thickness of the tectal layers, variations in axon diameter, in the extension and density of the terminations of individual axons and in the rostrocaudal position of the different projections also influence these results (Grover and Sharma, '81). It may be concluded that several gross features of the laminar organization have become clear recently (see fig. Vb), but that many experiments still have to be performed to obtain a detailed insight in the afferent pattern of the tectum of teleosts.

1.4 Quantitative aspects

From a quantitative point of view the retinal fibers constitute by far the most important tectal afferent system in the goldfish. From countings published by Schellart ('73) a total number of about 200.000 retinal ganglion cells can be calculated for a goldfish of about 20 cm length, and by far most of these project to the tectum (see above). A similar number of optic fibers was determined for *Eugerres plumieri* (Tapp, '74). Retinal fibers make about 30 million synaptic contacts in the goldfish (chapter IVb).

As far as the goldfish is concerned, afferents from the torus longitudinalis are quantitatively the second in importance. The tectum is the only known target of the torus longitudinalis (Ito and Kishida, '78; Vanegas et al., '81), and the importance of the toro-tectal projection can be judged from the number of cells in the torus, the thickness of the marginal layer, and from the number of synapses in the marginal layer. For goldfish the number of toral cells is about 100.000 (derived from cell counts in sections stained with cresyl violet) and the number of synapses made by the marginal axons is about 2.4 million (see the synaptic contacts of type I cells in chapter IVb). Among different teleosts large differences may occur in the relative thickness of the optic (layers 5 and 6) and marginal (layer 7) layers (Kishida, '79). The marginal layer attains its largest thickness in *Holocentrus* (Kishida, '79; Schroeder et al., '80) whereas it is smallest in *Trachinocephalus* (Kishida, '79). The optic layers have their largest relative thickness in the teleost *Navodon* (Kishida, '79; Ito et al., '81). The goldfish has a "standard" tectum in this respect (Kishida, '79).

Quantitative data concerning the number of axons and synaptic contacts of other tectal afferent systems are not available at present. Only the following can be noticed. The telencephalic projection might well be the third in importance, considering the rather large dimensions of the area centralis dorsalis, the large number of cells labeled after tectal HRP injections (Ito and Kishida, '77) and the dense degeneration in the SGC after telencephalic lesions (Vanegas and Ebbesson, '76). The nucleus isthmi probably has a rather heavy projection to the tectum as well (Sakamoto et al., '81). In contrast, the intertectal projection is reported to be very sparse (Ebbesson and Vanegas, '76; Sligar and Voneida, '76; Grover and Sharma, '79, '81; Ito et al., '80; Luiten, '81), and also only a small percentage of cells in the torus semicircularis seems to project to the tectum (Grover and Sharma, '81; Luiten, '81). The heaviness of the remaining projections is uncertain. Judging from the small size of the nucleus pretectalis, area pretectalis, nucleus dorsolateralis and nucleus of the rostral mesencephalic tegmentum (figs. V.4 and V.5), these centers probably give rise to only small numbers of tectal afferents.

2 INTRATECTAL CONNECTIVITY

2.1 Tectal cytoarchitecture

After the discussion dealing with the literature on tectal afferents, our attention will be focussed now on the tectal neurons. Apart from our own study (chapter III), the morphology of tectal neurons in the goldfish has been described by Leghissa ('55) and Romeski and Sharma ('79). Whereas a discussion of the results of Leghissa ('55) has already been incorporated in chapter III (p. 46-49, table 2 on p.47), the study of Romeski and Sharma ('79), published later, should be briefly discussed here.

Comparison of the study of Romeski and Sharma ('79) with our reveals several differences. Our type II, V, VI, VII, VIII and XI neurons are not described by Romeski and Sharma ('79), the remaining cell types are classified in a different way, and Romeski and Sharma ('79) give no quantitative data, neither for the number of neurons investigated, nor for the somatic, dendritic and axonal characteristics of the neurons presented, nor for their frequency of occurrence. Furthermore, several types of neurons seem to have deviating axonal properties. E.g., Romeski and Sharma ('79) describe a horizontally running axon for type XII or fusiform neurons, whereas we could clearly identify a recurrent, efferent axon for this cell type. However, since it is not clear which criteria were used by Romeski and Sharma ('79) for the identification of axons, these differences cannot be evaluated in detail. Except for the differences mentioned, the morphology of the majority of neurons reported by Romeski and Sharma ('79) is in substantial agreement with that of the cell types described in the present thesis.

Tectal cytoarchitecture has also been analysed with the Golgi-technique in the teleosts *Barbus fluviatilis* (Ramon, 1899), *Salmo irideus* (Bathelt, '70; Pinganaud and Clairambault, '79), *Eugerres plumieri* (Vanegas et al., '74), *Bagrus sp* and *Ictalurus punctatus* (Schroeder and Vanegas, '77), *Hemichromis bimaculatus* (Coss and Globus, '79), and *Holocentrus rufus* and *H. ascensionis* (Schroeder et al., '80). The first four of these studies have been discussed in chapter III (table 2, p. 47). The similarity between the results of Vanegas et al., '74 and ours was stressed, and it was concluded that, except for some quantitative differences, the tecta of the teleosts studied up to that time were basically similar. This conclusion has been supported by the comparative study of Kishida ('79) on a large variety of teleosts and also by the other recent Golgi-studies mentioned above.

Whereas several Golgi-studies on the teleostean tectum tend to stress the variability of the individual neurons (Leghissa, '55; Vanegas et al., '74; Romeski and Sharma, '79; Schroeder et al., '80), others tend to stress a strict classification of cell types on basis of relevant similarities (present thesis, chapter III; Kishida, '79). The schematic presentation of cell types in figure 19, p. 51, is the result of the latter approach, presenting a classification of cell types with averaged characteristics determined in a quantitative way (see chapter III). Such a presentation, which like all classifications necessarily includes some simplifications, neglects several variabilities and favours some characteristics at the expense of others, might seem to be simplistic in view of the capriciousness of the Golgi-methods and the large variability of individual neurons observed. It should, however, be realized that (1) When a large number of neurons is investigated with a large variety of Golgi-modifications (see section III), a rather complete description of neuronal types may be obtained; (2) The variability of tectal neurons sometimes is more apparent than real because of incomplete impregnation, oblique planes of sectioning and difficulties in defining tectal layers in Golgi-material; (3) The method employed for classification (see fig. 2, p.34) clearly shows monomodal distributions for the location of specific dendritic trees; and (4) E.M. investigation has revealed that at least six of the cell types distinguished by means of this classification have characteristic synaptic properties as well (chapter IV.b).

2.2 Laminar organization of the tectal neuronal elements

The present knowledge concerning the stratification pattern of both afferents and cell types in the goldfish tectum is summarized in figure V.7. With respect to the tectal afferents, the main results as discussed in section V.1 are summarized in this figure (cf. fig. V.6). With respect to the tectal cell types, figure V.7 is derived from figure 19 on page 51. However, to stress the stratification pattern of the tectal cell types, only the average location of their dendrites and axons is presented, whereas information about tectal cell bodies, which do not seem to have any specific significance in tectal circuitry deviating from that of dendrites (chapters III and IV), as well as information about dendritic and axonal extensions is omitted. Figure V.7 reveals the following organizational features of the tectum:

2.2.1 Tectal afferents

Tectal afferents are localized in four distinct zones:

1) Afferents from the torus longitudinalis terminate in layer 7, which thus can be characterized as the toral afferent layer. Since the torus longitudinalis gets its signals predominantly from the valvula cerebelli (Ito and Kishida, '78), layer 7 may also be called the cerebellar afferent layer (Schroeder et al., '80).

2) Layer 5 and its border zones (layer 5/6 and 4/5) contains the bulk of retinal afferents, and in addition afferents from the nucleus pretectalis, the area pretectalis, the nucleus isthmi and the nucleus of the rostral mesencephalic tegmentum. The nucleus pretectalis and area pretectalis can both be considered as "visual" nuclei, since they have an important bilateral input from the retina (fig. V.1). The nucleus isthmi may also be considered as a "visual" nucleus, since it receives its input from the "visual" nucleus pretectalis (Ito et al., '81b) and from a subtype of type XIV cells (Ito et al., '81b), which also must be considered as "visual" (see below). So, layer 5 and its boundaries may be designated as the "visual" afferent zone, not only because of their retinal input, but also because of the presence of afferents from the nucleus pretectalis, area pretectalis and nucleus isthmi. The nucleus of the rostral mesencephalic tegmentum provides signals of unknown modality to this zone.

3) The bulk of telencephalic afferents terminates in the SGC, particularly

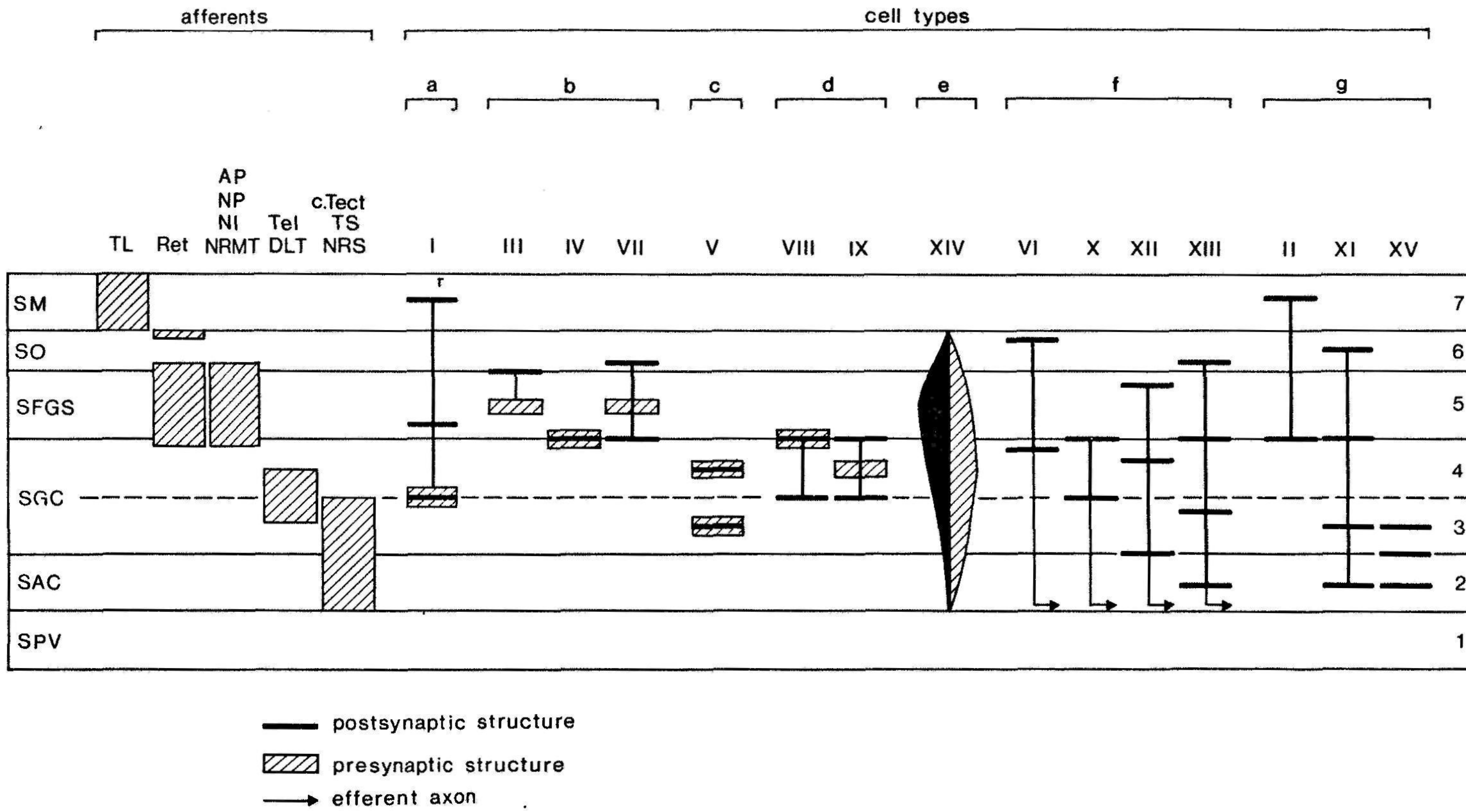


Fig. V.7. Summarizing scheme of the laminar organization of the tectal neuronal elements. With respect to the tectal afferents, the main results as described in section 1.3 and summarized in fig. V.6. have been indicated. With respect to the tectal cell types, the average position of the dendritic trees and axon terminals has been indicated (without indication of their extension), as described in chapter III and summarized in fig. 19, pag. 51 (see also fig. C of the SAMENVATTING). For further details see text section 2.2. For abbreviations: see pag. 110.

in layer 3/4, which thus could be considered as the telencephalic afferent zone However, also the axons of type I neurons, which are the recipients of toral signals, terminate in layer 3/4. Consequently, this zone should preferably be considered as the telencephalic-toral (cerebellar) afferent zone. In addition, axons of the nucleus dorsolateralis tegmenti probably terminate in this zone

4) Afferents from the contralateral tectal half, torus semicircularis and reticular formation specifically terminate in layers 2 and 3, which constitute the "deep" afferent tectal region. Auditory as well as lateral line responses recorded from the tectum are probably provided via the afferents in this layer. In addition, some retinal fibers terminate in layer 2, while also the area pretectalis, nucleus pretectalis, nucleus isthmi and nucleus of the rostral mesencephalic tegmentum might have some additional projections to these layers (see fig. V.6). It should be mentioned that the present study (chapter IVa) revealed at least three types of myelinated afferents in this zone.

2.2.2 Tectal interneurons

By relating the dendrites of tectal interneurons to the afferent zones described above, five groups can be distinguished (fig. V.7):

(a) Interneurons with their dendrites mainly in the toral (cerebellar) afferent layers (type I neurons).

(b) Interneurons with their dendrites in the visual afferent layer (type III, IV and VII neurons).

(c) Interneurons with their dendrites in layers 3 and 4 (type V neurons).

(d) Interneurons with their dendrites both in the "visual" as in the telencephalic-toral (cerebellar) afferent layer 3/4 (type VIII and IX).

(e) The less strictly organized and very numerous type XIV neurons, which have their main dendritic tree in the visual afferent layer 5, and additional dendrites in other tectal layers.

With respect to the axon terminations of tectal interneurons, figure V.7 shows a rather refined stratification pattern in comparison with the pattern of tectal afferents. The axons of type I neurons specifically terminate in layer 3/4. The axons of the interneurons of group b, c and d terminate in layer 5, 4/5 or 4 in such a way that each of these layers contains the axon terminals of one monostratified or horizontal neuron and one bistratified neuron (layer 5: type III and type VII; layer 4/5: type IV and type VIII; layer 4: type V and type IX). A portion of type V neurons is located in layer 3, but otherwise the "deep" afferent layers are very poor in axon terminations of interneurons, in contrast to the more superficial layers 4 and 5. The axons of type XIV neurons, which individually may show many variations, on the average terminate in layer 5, 4 as well as 3, with most terminals in layer 4, the only tectal zone in which most probably no afferent terminals occur.

2.2.3 Efferent tectal neurons

Comparison of efferent neurons (group f in fig. V.7) with tectal interneurons reveals that type VI neurons show some correspondence with group b in having their dendrites in or near the visual afferent layer, and that type X neurons show a close correspondence to group d with dendrites in layer 4/5 as well as in layer 3/4. Type XII and XIII constitute a separate group of multistratified efferent neurons.

It should be noticed that three tectal cell types have obscure axonal properties. These are indicated as a separate group in figure V.7 (group g).

2.3 Functional implications of tectal cytoarchitecture and synaptology

In the next paragraphs an attempt will be made to analyse the significance of the structural features outlined above (fig. V.7) for the processing of signals from the four main streams of tectal input: (A) toral (cerebellar) input; (B) visual input; (C) telencephalic input and (D) "deep" input. This analysis comprises both a qualitative and a quantitative part.

The qualitative part of the analysis starts from the following facts and assumptions:

(1) The stratification pattern of tectal afferents and cell types as presented in figure V.7 and discussed in the preceding sections.

(2) The assumption that all neurons belonging to a certain tectal cell type as distinguished in the present thesis (fig. 19, p. 51 and fig. V.7) have a similar function. Synaptic evidence for this assumption has been presented and discussed in chapter IVb.

(3) The finding that dendrites, dendritic shafts and cell bodies in the goldfish tectum are exclusively postsynaptic or signal-receiving structures, whereas axon terminals are exclusively presynaptic or signal-providing structures (see chapter IVa).

(4) The assumption that all types of presynaptic structures at a certain level form synaptic contacts with all types of postsynaptic structures at the same level. This assumption will be discussed in detail in section V 2.3.6.

The procedure followed in the quantitative part of the analysis may be outlined as follows (details are presented in the *APPENDIX*): The relative importance of the various types of postsynaptic structures is estimated on the basis of the numbers of cells per cell type (presented in chapter III) combined with the numbers of synapses (presented in chapter IVb; fig. 4, p. 98; type I, VI, XII, XIII and XIV) or, when these numbers are not available, on the basis of the extensions of the different postsynaptic components given in chapter III (table 3, p. 50; type VII, VIII, IX, X, XI and XV). With respect to the relative importance of different types of presynaptic structures occurring at the same tectal level, the following additional assumptions have been introduced:

(5) All types of postsynaptic structures at a certain tectal level make equal percentages of synaptic contacts with all types of presynaptic structures present at the same level, except for the axons of type XIV cells, which are considered separately because of their large number and diffuse stratification pattern.

(6) With respect to type XIV neurons, primarily two conditions will be considered in the next paragraphs: (a) At first tectal circuitry will be analysed assuming that type XIV axons exclusively make synaptic contacts with type XIV dendrites, and consequently have no influence on the other types of tectal neurons. (b) Secondly, this condition will be compared with a condition in which type XIV axons make a large number of contacts with all of the other tectal cell types (for precise numbers, see *APPENDIX*). As will be discussed (section 2.3.6), the reality can be expected to lie somewhere in between condition (6) a and (6) b. For the sake of simplicity, it is furthermore assumed that:

(7) The influence of a certain axon on a certain neuron is proportional to the number of synaptic contacts between both structures.

The assumptions (5), (6) and (7) will be discussed in detail in section V 2.3.6. The next paragraphs are illustrated by figures V.8 to V.11, the design of which is basically similar to figure V.7, with the following exceptions: (1) the axons have been drawn as lines, (2) some information about the dendritic extension and frequency of occurrence of the cell types in included and (3) type XIV neurons have been drawn in a special way because of their peculiar role in tectal circuitry.

2.3.1 Toral input processing

The direct input from the torus longitudinalis to the tectum is exclusively received by the apical dendrites of type I neurons (fig. V.8). Type II neurons have processes in layer 7 as well, but there is good evidence for *Eugerres* and *Holocentrus* that these processes are presynaptic (Vanegas et al., '79; Schroeder et al., '80; Ito et al., '80). Their low frequency of occurrence in the goldfish tectum might explain why these structures were not observed in our own EM study (chapter IV).

Judging from the distribution of synapses on type I neurons, their axonal signals contain at least 90% toral information. These signals are conveyed to postsynaptic structures in layer 3/4, which include the basal dendritic tree of type VIII, type IX and type X neurons, cell bodies of type IX and type X neurons, and dendritic shafts of type XII, type XIII and type XIV neurons (fig.V8). Some of the type XIV neurons may have considerable dendritic trees in this layer as well. Thus, the most important candidates for further processing of the toral information are type VIII, type IX and type X neurons. Type VIII and type IX neurons are interneurons, which bring about an upward transport of information from layer 3/4 to layer 4/5 and 4 respectively. From layer 4/5, a further upward transport to layers 5 and 5/6 can be effected by the axons of type VII neurons. At this tectal level, however, the relative importance of toral information is probably very low (fig. V.8). A large amount of synaptic contacts between the axons of type XIV neurons and the other cell types (see assumption (6)b, above) would decrease the relative importance of toral signals in tectal circuitry, since type XIV neurons are in general no important targets of type I axon terminals (fig. V.8 and V.12).

The fastest route from the toral input in layer 7 to tectal output is a two-synaptic pathway via type I and type X neurons. The axonal signals of type X neurons, however, do probably not contain more than 30% toral information. The other tectal efferent neurons are to a still lower extent influenced by toral signals, while this influence is provided for a large part by pathways involving three or more synapses (fig. V.8).

So, the processing of toral information in the tectum has a number of very peculiar characteristics. No tectal efferent neuron is directly contacted by toral afferents. All toral information is received by one specific type of interneuron , which conducts toral information downwards to one specific layer (layer 3/4). In this layer, again interneurons constitute most of the post synaptic structures, which, in turn, effect an upward transport of toral information. Only one efferent neuron (type X) has dendrites specifically in layer 3/4, but it does probably not send more than 30% toral information outside the tectum. Consequently, there is a discrepancy between the large amount of toral input to the tectum and the low degree of representation of this input in the tectal outflow.

2.3.2 Visual input processing

As described above, the layers 5/6, 5 and 4/5 receive visual input from the retinal fibers as well as by some afferents from the nucleus pretectalis, the area pretectalis and the nucleus isthmi. Although these layers may also receive a small amount of non visual afferent signals, for the sake of simplicity the input of layers 4/5, 5 and 5/6 (the SFGS) will be considered as 100% visual in the following discussion. The visual afferents most probably make synaptic contacts with all tectal cell types except for types V and XV, the only cell types without dendrites in the visual afferent layer. Retinal afferents constitute about 18% of the presynaptic terminals in the SFGS (Airhart and Kriebel, '80), an average value which is comparable to the values presented in this thesis for five specific cell types (types I, III, VI, XII and XIV). Therefore, it seems likely that all postsynaptic structures in the SFGS receive about the same degree of direct visual input (fig. V.9).

Type III, IV and VII neurons may be considered as specific visual interneurons, since they have their postsynaptic structures exclusively in the visual afferent layer. They may also process some non visual information by way of contacts with the axons of type VIII neurons (type IV and VII) or of

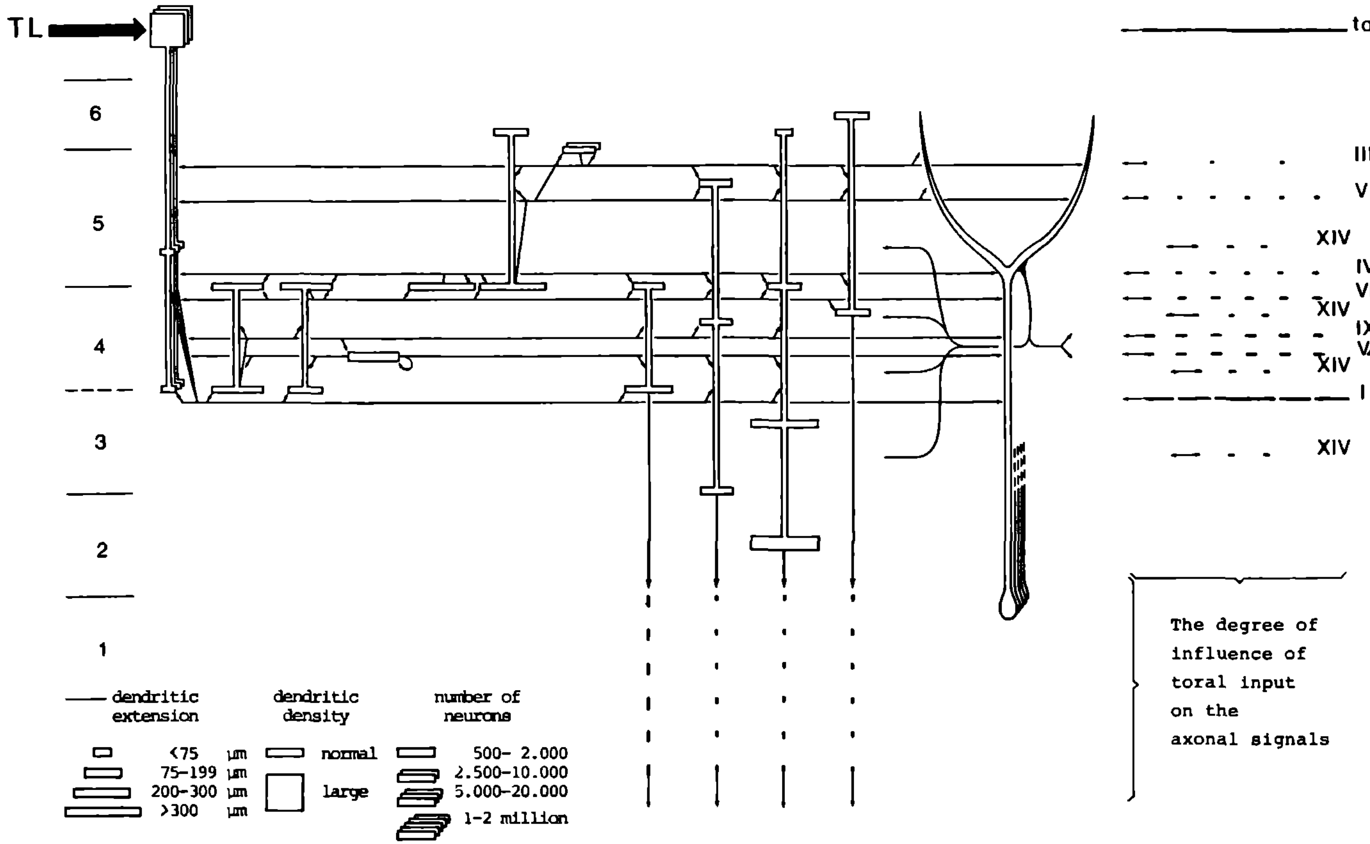


Fig. V.8. The tectal circuitry involved in the processing of toral input. Dendritic shafts are indicated by vertical bars, dendritic trees by horizontal bars. These are located as in fig. V.7. Axons are indicated by lines, also located as in fig. V.7, and (supposed) synaptic contacts by arrows. The dendritic extension, the dendritic density and the number of neurons per tectal half are encoded as indicated left below in the figure. The cell types have been placed in a rough sequence of importance. The cell groups indicated are discussed in section 2.2.2. The influence of toral input on the axonal signals is indicated as calculated in the appendix for condition 1. Completely black lines indicate 100% influence, white lines would indicate 0% influence. For further details, see general discussion section 2.3.1.

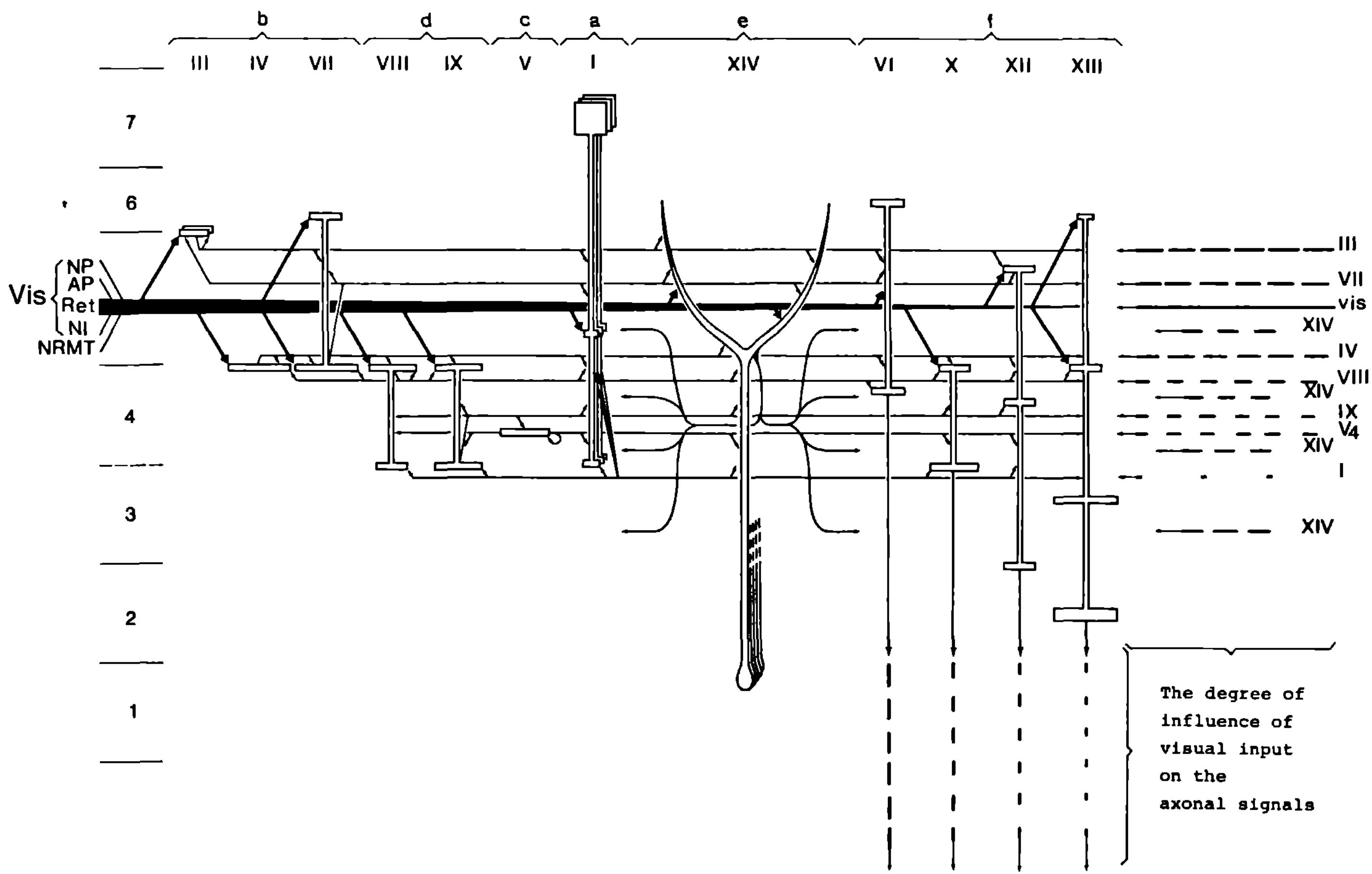


Fig. V.9. The tectal circuitry involved in the processing of visual input. For details, see the legends of fig. V.8 and general discussion section 2.3.2.

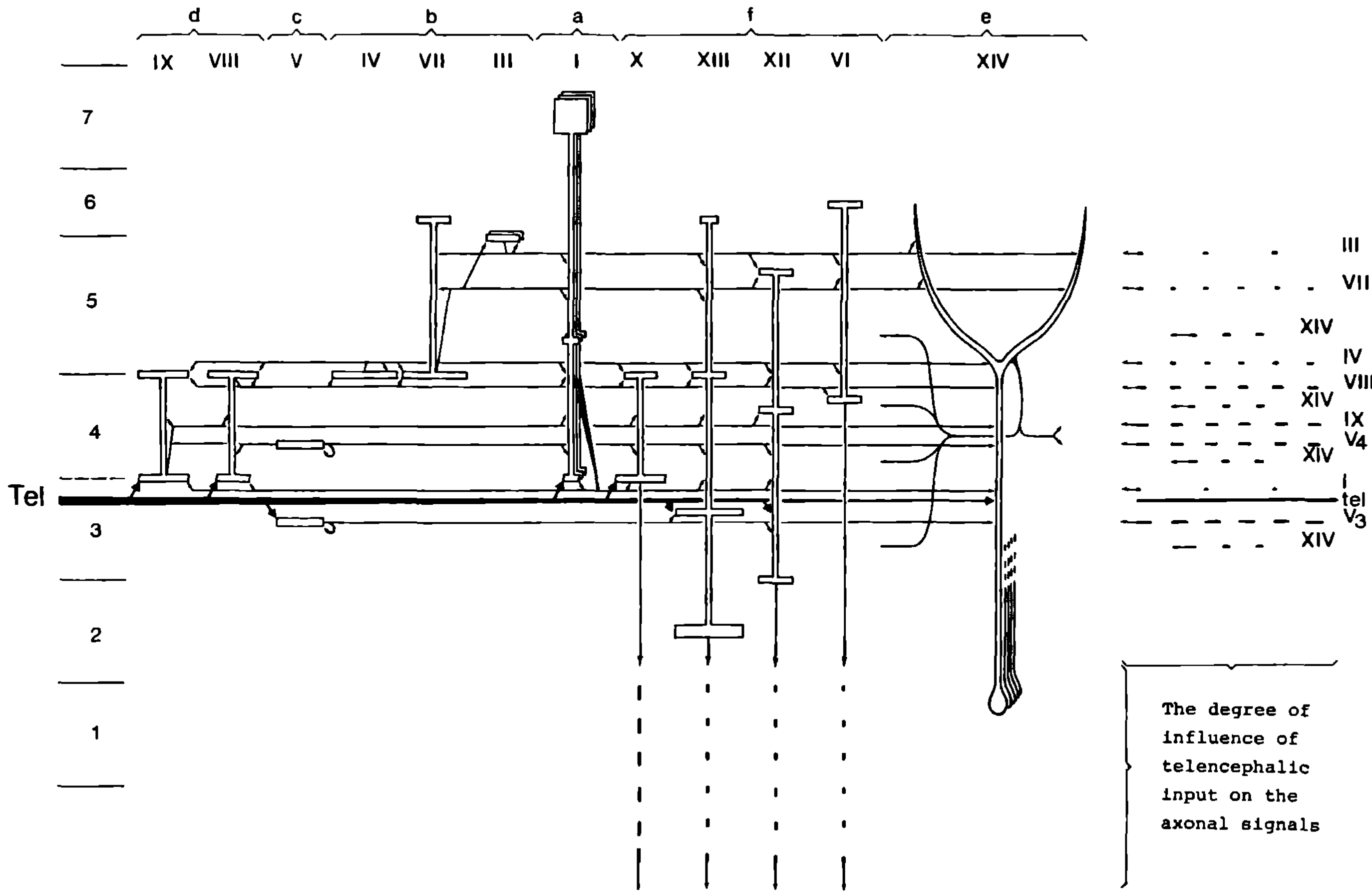


Fig. V.10. The tectal circuitry involved in the processing of telencephalic input. For details, see the legends of fig. V.8 and general discussion section 2.3.3.

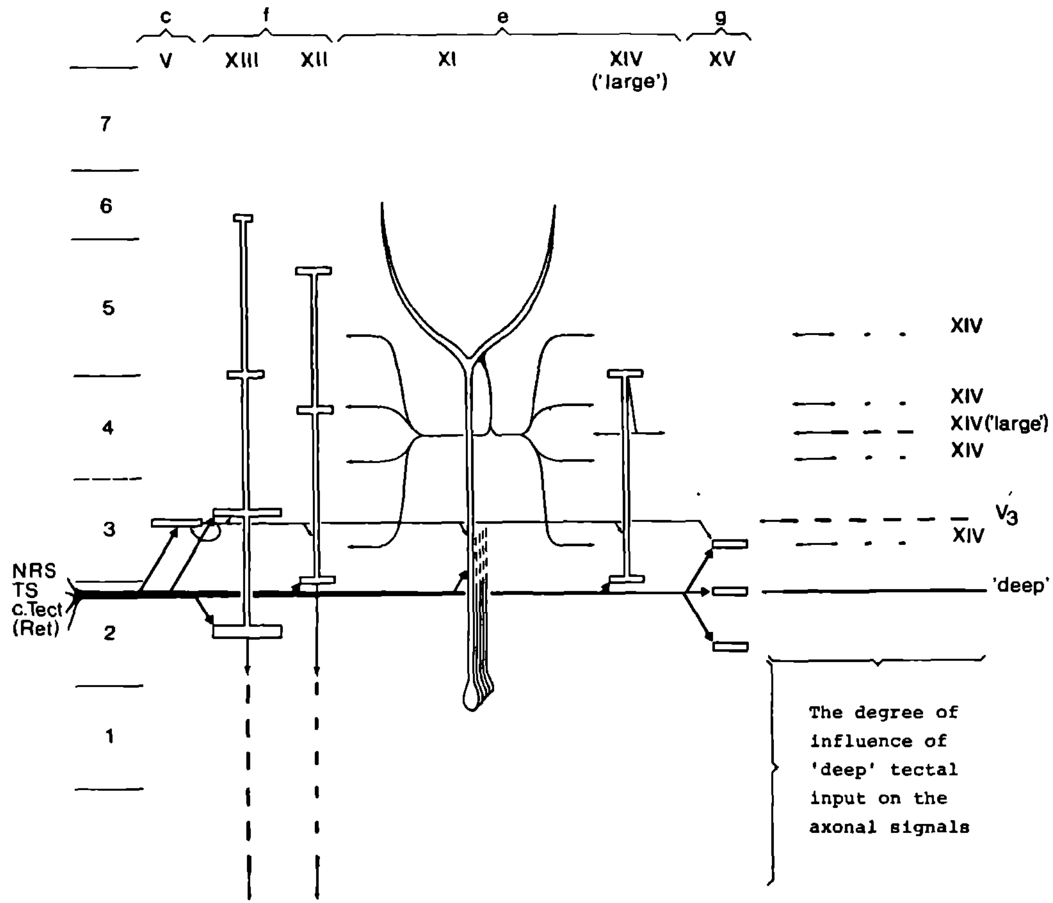


Fig. V.11. The tectal circuitry involved in the processing of 'deep' tectal input. For details, see the legends of fig. V.8 and general discussion section 2.3.4.

type VII neurons (type III), but this is probably only a low percentage (fig. V.9 and V.13). The axons of type III and of type VII neurons terminate in layer 5 or 5/6, where no other interneurons have axon terminals, except type XIV, which will be discussed below. This stresses the importance of this layer for visual information processing, since not only the visual afferents appear to terminate in layer 5, but also the axons of the visual interneurons type III and type VII. Type IV neurons have their axon terminals in layer 4/5. Apart from the visual afferents, layer 4/5 also contains the axons of type VIII neurons, providing some non-visual information from deeper tectal levels (fig. V.9). So, layer 4/5 is somewhat less "visual" than the remaining part of layer 5. Layers 4 and 3/4 may only receive "indirect" visual input via the axons of type IX and type I respectively, which bring about a downward transport of visual information. As can be judged from the number of synaptic contacts in layer 5, type I neurons probably do not receive more than 5% visual information in the goldfish, whereas type IX would receive about 40% visual information (fig. V.13).

Type XIV neurons would process for about 60% visual information under both conditions assumed in fig. V.13. The main effect of a large number of contacts between axons of type XIV neurons and dendrites of other cell types would be a strong downward flow of visual information from SFGS to SGC, where type XIV axons have many terminals (figs. V.7 and V.9). This, in turn, would in particular enlarge the influence of visual information on the tectal efferent neurons (fig. V.13).

Most likely all types of efferent tectal neurons have synaptic contacts with visual afferents (fig. V.9). For type VI and type XII this has been shown in the electron-microscope (chapter IV). Type VI neurons are probably the most "visual" efferent neurons, since they are rather strictly located in the visual afferent layer. The position of the dendritic trees of type VI cells raises some questions, however. The present ultrastructural study (chapter IV) revealed that their apical dendritic tree, preferentially located in the superficial part of layer 6, is not contacted by retinal fibers. Since interneurons equally have no axon terminals in this region, except for some type XIV neurons, it is not clear which presynaptic structures make contact with the apical dendritic tree of type VI neurons. Possible candidates are the retinal terminals that constitute the thin superficial band of the retino-tectal projection (fig. V.7), which, then, should have ultrastructural characteristics deviating from the bulk of the retino-tectal fibers. Other candidates are fibers from the nucleus pretectalis, area pretectalis, nucleus isthmi or nucleus of the rostral mesencephalic tegmentum, which, then, should terminate specifically in the superficial part of layer 6. It is important to notice that Laufer and Vanegas ('74b) have described large axons at this tectal level which do not degenerate after eye-enucleation. Although Laufer and Vanegas ('74b) considered these fibers as tectal efferents, it is equally conceivable that they constitute non-retinal afferents. Disregarding these uncertainties, the apical dendrites of type VI neurons are assumed to receive 100% visual input (figs. V.9 and V.13).

The basal dendritic tree of type VI neurons, preferentially located in layer 4 just below layer 4/5, also makes no synaptic contacts with terminals identified as retinal fibers (chapter IV). Although it might be possible that the axons of type IV, type VII and type IX neurons make some synaptic contacts with the basal dendritic tree of type VI, most probably type XIV axons make most of the synaptic contacts on this dendritic tree. This would be in line with the high frequency of occurrence of type XIV axons in this layer and with the small size of the synaptic contacts of the basal dendrites of type VI cells (chapter IV). However, this implies that, at least for layer 4, an assumption that type XIV axons exclusively make synaptic contacts with type XIV dendrites, is not realistic.

The axons of type X, XII and XIII would at least transfer about 40%, 25% and 15% visual information outside the tectum, respectively (fig.V13). Most of this visual information reaches these neurons via bi- or multi synaptic pathways (fig. V.9) although all these tectal efferent neurons constitute monosynaptic pathways between visual input and tectal output as well. The percentages enumerated increase with increasing involvement of the axons of type XIV neurons in tectal circuitry (fig. V.13).

Summarizing, visual input processing in the tectum is about the contrary of toral input processing. Whereas toral input is received by a single type of neuron, visual input is directly provided to almost all tectal neurons; and whereas toral afferents do not contact efferent neurons directly, all efferent neurons have direct contacts with visual afferents. A further contrast between toral and visual input processing is the simplicity of the toral afferent layer 7 (only one type of presynaptic element and one type of postsynaptic element) and the complexity of the visual afferent layer (layer 5 and its boundaries), where at least nine types of presynaptic elements and thirteen types of postsynaptic elements are involved in tectal circuitry. A further characteristic of visual input processing is the occurrence of several types of visual interneurons which make synaptic contacts close to the contacts made by the visual afferents, an arrangement not realized for the toral input. Visual input has a relatively high influence on the tectal outflow, whereas the toral influence seems low in this respect (fig. V.13).

2.3.3 Telencephalic input processing

There is a large degree of similarity between the processing of telencephalic and toral information, since telencephalic afferents terminate at the same tectal level (layer 3/4) to which type I axons convey their toral information (cf. figs. V.8 and V.10). Accordingly, just as has been discussed for toral input (section 2.3.1), some part of the telencephalic input to layer 3/4 is transferred upward via the axons of type VIII, IX and VII (fig. V.10), and the most important type of efferent neuron for telencephalic information is probably type X. A large influence of type XIV neurons would likewise decrease the influence of telencephalic input on the tectal outflow (fig. V.13).

The largest differences between toral- and telencephalic input processing are the involvement of type I neurons and layer 7 in toral information processing, and the fact that telencephalic afferents are probably less strictly concentrated in layer 3/4 than the axons of type I neurons. Consequently, in contrast to the situation for toral input, there does exist a monosynaptic pathway from the telencephalic input to the tectal output via type X neurons and probably also via type XII and type XIII neurons. Of the latter two, type XIII neurons might be the most important one in this respect, since their dendrites in layer 3, preferentially located just below layer 3/4, might well have synaptic contacts with telencephalic afferents (fig. V.10). Type XII neurons reveive most of their telencephalic information via contacts of their dendrites in layer 4 with axons of type IX. However, for the same reasons as discussed above for the dendrites of type VI neurons in layer 4, type XIV axons probably constitute most of the presynaptic elements of the dendrites in layer 4 of type XII neurons. This would reduce the telencephalic influence on type XII neurons substantially (see fig. V.13).

The ratio between telencephalic- and toral input in layer 3/4 is not clear, since the number of telencephalic afferents, as well as the number of contacts made by these afferents is unknown. Under the conditions assumed in the foregoing discussion, i.e. the same degree of importance of different types of presynaptic elements in a particular layer, telencephalic input would be somewhat more important than toral input because of the involvement of type I neurons, but this is merely hypothetical. What is important, however, is the competing character of telencephalic and toral (cerebellar) afferent system in tectal circuitry. The larger the influence is of one of the two, the smaller the influence of the other will be, since both have to be integrated to a large extent by the same postsynaptic structures.

It should be mentioned that afferents from the mesencephalic nucleus dorsolateralis also terminate in the SGC. For the sake of simplicity, however, these will be left out of discussion. The importance of this afferent system is obscure at this moment.

2.3.4 "Deep" tectal input processing

The so-called "deep" tectal afferents, including fibers from the contralateral tectum, the torus semicircularis, and the reticular formation, terminate almost exclusively directly on the large tectal efferent neurons, type XII and type XIII, which have large dendritic trees in the deep afferent layers 2 and 3 (fig.V.11). Interneurons hardly occur in these layers,except for some type V neurons in layer 3 which therefore can be considered as interneurons for the deep afferent input and the dendritic shafts of type XIV neurons. So, in this respect the deep tectal input processing seems to be the opposite of the toral input processing, the deep tectal afferents terminating predominantly on efferent neurons, and toral afferents terminating exclusively on interneurons. Visual and telencephalic input processing take an intermediate position in this respect, since visual and telencephalic afferents terminate on both interneurons and efferent neurons. A large number of contacts of type XIV axons with all tectal cell types would result in a substantial upward transport of deep tectal input to layers 4 and 5 and would increase the influence of deep tectal input on tectal interneurons as well as efferent tectal neurons (fig. V.13).

The deep input processing in the tectum might, however, be more complex than suggested above for the following reasons. Firstly, type XV neurons, a population of neurons with dendrites predominantly in layer 2 and 3, are not included in the preceding discussion because their axonal properties are obscure. Nevertheless, they will be involved in the deep input processing because of their dendritic properties. A portion of type XV neurons might well be interneurons with axons terminating in the tectum, but others give rise to tectal efferents, as was indicated by Romeski and Sharma ('79). Secondly, in particular the so-called "large" subtype of type XIV neurons (chapter III) may have considerable dendrites in layer 2 and/or 3. Consequently, these cells might have a still more important function in the integration of visual and "deep" tectal input than the bulk of type XIV neurons (fig. V.11). In chapter III as well as in the publication of Romeski and Sharma ('79) these large type XIV neurons are considered as interneurons, just as the other type XIV cells.

2.3.5 Functional considerations concerning the tectal layers and cell types

In the previous sections of this chapter the distribution of information in the tectum provided by its four main streams of input has been discussed. From the data presented the following stratification pattern emerges (fig. V. 12). The four levels of tectal input (see section 2.2.1) remain clearly distinguishable. However, in the interjacent layers a considerable mixing of the different modalities is brought about by the tectal interneurons, in particular in layer 3 and in layer 4 (fig. V.12). Only the boundary between layer 7, the toral input layer, and layer 6, the visual input layer is very sharp. Consequently, layer 7 can be considered exclusively as a relay station for toral (which means cerebellar) input to the tectum, with no other relations with the deeper tectal layers than via the apical dendrites of type I neurons. In this respect, type I neurons could equally well be considered to be tectal afferents as to be tectal interneurons. Another sharp boundary can be observed between layer 2 and layer 1, the stratum periventriculare, which contains exclusively cell-bodies with very few synaptic contacts.

The remaining part of the tectum, layers 2-6, is constituted by closely

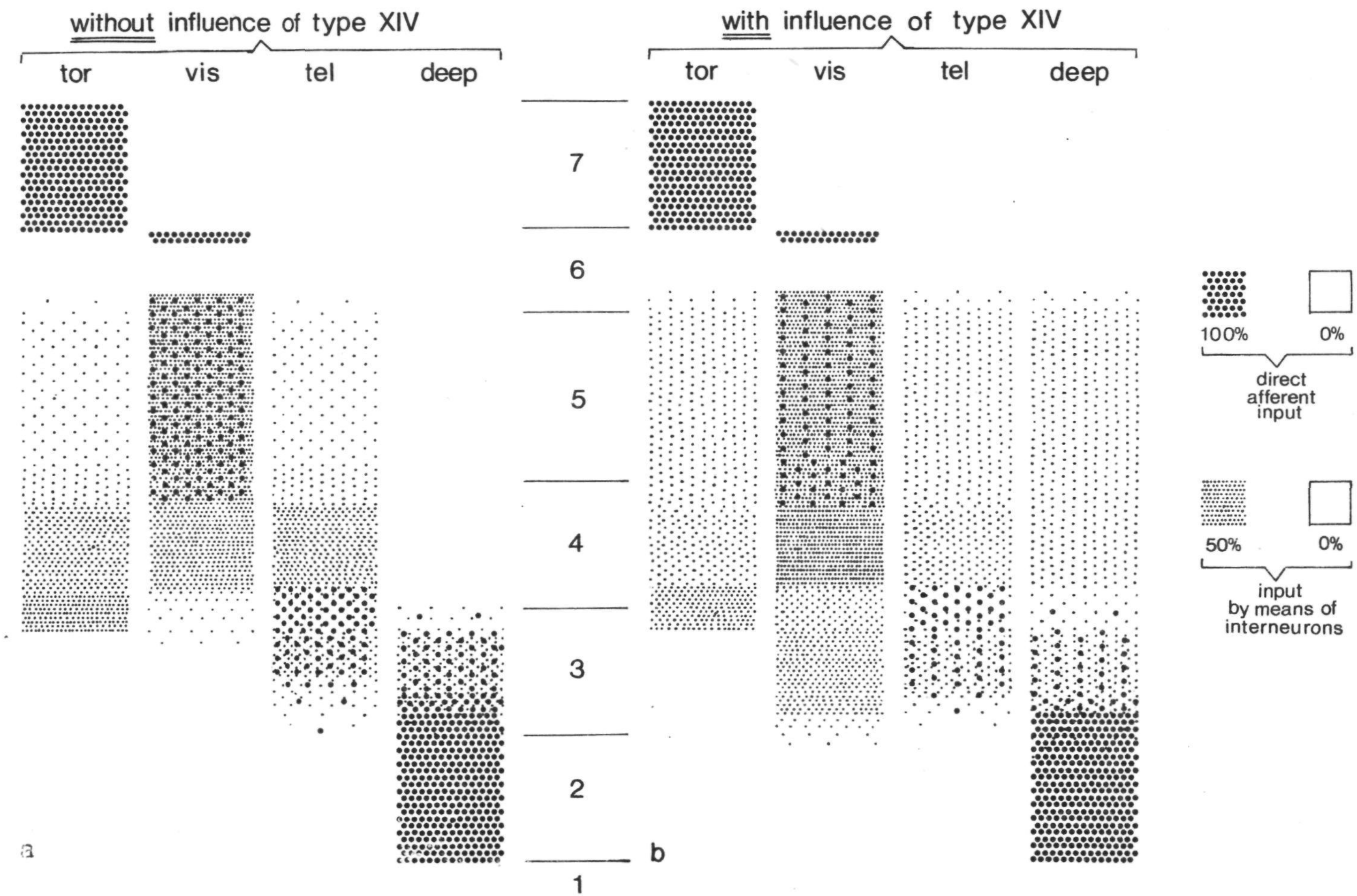


Fig. V.12. Summarizing scheme of the percentiv influence of the four main streams of tectal input in the various tectal layers, both direct by means of afferents and indirect by means of interneurons. a: as calculated in the appendix for condition 1 by menas of the formulae 14a-21a and 14b-21b; b: as calculated in the appendix for condition 3 by menas of the formulae 14d-21d and 14e-21e.

interrelated layers with only gradual transitions from one layer to another. The superficial part, layers 6 and 5, is predominantly involved in visual information processing (fig. V.12) whereas the deep layer 2 is almost exclusively involved in "deep" information processing. In layer 3 a considerable mixing of visual and "deep" input might occur by means of the action of type XIV neurons (cf. fig. V. 12a and b). In layer 3/4, just in between the deep non-visual and the superficial layers, the telencephalon and the cerebellum exert their influence, the latter after interposition of the torus longitudinalis. In layer 4 some degree of mixing between visual, telencephalic and toral information is realized. The importance of visual information in this layer strongly depends on the involvement of type XIV cells (cf. fig. V.12a and b). Layer 4/5 is remarkable because of the large extension of several dendritic trees, suggesting an important function in spatial integration of visual input.

The functions of the various tectal cell types have already been discussed in some detail in the preceding paragraphs. In summary, the following can be noticed (see fig. V.13).

Type I neurons may be considered as the tectal afferents for toral (cerebellar) input. In addition, they process some amount of visual input (via dendrites in layer 5) and telencephalic input (via dendrites in layer 3/4).

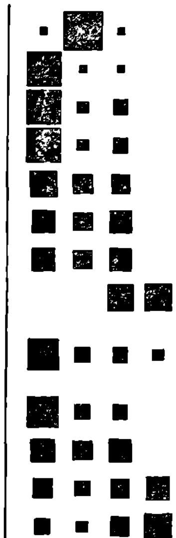
Of the visual interneurons type III, IV and VII, type IV and VII have very extensive dendrites which make them suitable for a function in spatial interaction and integration of visual signals. The location of their dendrites in layer 4/5 may also indicate a function in integrating visual input with some amount of telencephalic and toral input, which reaches layer 4/5 by means of the axons of type VIII neurons. The bistratified character of type VII may suggest that this type of neuron integrates more aspects of visual information than type IV and type III neurons. Type III neurons seem to be the most visual interneurons (fig. V.13) which is also suggested by their relative high percentage of contacts with retinal fibers (chapter IVb). Since their dendrites are not very extensive, they are more likely involved in temporal aspects of visual signal processing than in spatial aspects. The bulk of type XIV neurons can also be considered as visual interneurons, although for some subtypes other modalities may be important as well. Their large number with respect to the number of retinal and other afferents and their large variability make this cell type suitable for a number of specialized functions in signal processing.

Type V neurons, indicated as non-visual interneurons in figure V.7 constitute a heterogenous population, receiving different types of input depending on their position in layer 3 or 4. Their morphology suggests a function in spatial interaction between different tectal regions. Type XV might represent a second type of non-visual interneuron, involved in the processing of deep tectal input.

Type VIII and IX clearly integrate three different types of input: visual,

<u>witout</u> influence of type XIV

type	afferent input				input from interneurons										output						
	vis	tor	tel	deep	I	III	IV	VII	VIII	IX	V_4	V_3	XIV	form	VIS	TOR	TEL	DEEP	form	VIS TOR TEL DEEP	
I	2	90	2												5	90	5		(1b)		I
III	33					33		33						(2a)	88	6	6		(2b)		III
IV	33						33		33					(3a)	73	12	14		(3b)		IV
VII	33					7	27	7	27					(6a)	76	11	13		(6b)		VII
VIII	17		13		13		17		17	13	13			(7a)	47	25	29		(7b)		VIII
IX	13		20		20		13		13	10	10			(8a)	38	29	33		(8b)		IX
V_4										50	50			(4a)	38	29	33		(4b)		V_4
V_3			33	33								33		(5a)			50	50	(5b)		V_3
XIV	22α		7α	7α	6α	15α	7α	15α	7α	6α	6α	2α	100 x (1−α)	(9a)	60	14	18	8	(9b)		XIV
VI	33					4	4	4	54					(10a)	69	15	17		(10b)		VI
X	13		20		20		13		13	10	10			(11a)	38	29	33		(11b)		X
XII	5		8	33	8	3	2	3	2	14	14	8		(12a)	23	16	23	37	(12b)		XII
XIII	6		13	50	4	3	3	3	3	4	4	8		(13a)	17	7	21	54	(13b)		XIII
														⟶100%					⟶100%		



13a

with influence of type XIV

type	afferent input				input from interneurons										output									
	vis	tor	tel	deep	I	III	IV	VII	VIII	IX	V_4	V_3	XIV	form	VIS	TOR	TEL	DEEP	form	VIS	TOR	TEL	DEEP	
I	1	90	1										5		5	90	5	1	(1e)					I
III	17					17		17					50	(2d)	67	9	12	12	(2e)					III
IV	25						25		25				25	(3d)	70	9	12	9	(3e)					IV
VII	23					3	20	3	20				30	(6d)	69	9	12	10	(6e)					VII
VIII	13		8		8		13		13	3	3		40	(7d)	54	16	20	10	(7e)					VIII
IX	10		13		13		10		10	3	3		38	(8d)	47	20	25	9	(8e)					IX
V_4										13	13		75	(4d)	54	13	17	16	(4e)					V_4
V_3			17	17								17	50	(5d)	33	7	30	30	(5e)					V_3
XIV	13		5	7	4	7	6	7	6	1	1	1	38	(9d)	56	9	12	10	(9e)					XIV
VI	30					2	3	2	16				47	(10d)	70	9	12	10	(10e)					VI
X	10		13		13		10		10	3	3		38	(11d)	47	20	25	9	(11e)					X
XII	3		6	35	4	2	1	2	1	4	4	2	37	(12d)	32	10	15	44	(12e)					XII
XIII	4		7	46	3	1	2	1	2	1	1	4	28	(13d)	26	7	14	53	(13e)					XIII
														→ 100%					→ 100%					

13b

Fig. V.13. The percentive influence of afferents and axons of interneurons on the tectal cell types and the resulting influence of the four main streams of tectal input on the output of these cell types, as calculated in the appendix by means of the formulae indicated. In the right four columns of both parts of this figure the percentive influence is encoded by the surface of the squares. a: without the influence of type XIV axons (condition 1 of the appendix); b: with an influence of type XIV axons as described in condition 3 of the appendix.

telencephalic and toral input. Visual input seems the most important modality for type VIII, which has substantially larger dendrites in layer 4/5 than in layer 3/4, whereas for type IX the toral/telencephalic input is more important. This tendence is stressed when the position of the cell body and the axon origin is taken into consideration.

Among the efferent neurons, type VI neurons are probably specialized to transmit visual information beyond the tectum. Their receptive surface may integrate all aspects of visual information processing from layer 6, 5 and 4. Type X neurons are in particular important for transport of toral and telencephalic information out of the tectum, integrated with visual information. The large efferent neurons of type XII and type XIII integrate information from all tectal input systems. For type XIII neurons, however, the deep tectal input is the dominant modality (fig. V.13), the importance of which is stressed by the location of the cell body and axon origin of type XIII neurons in layer 2. For type XII neurons the visual input is relatively more important than for type XIII neurons. The ratio between the influences of the distinct types of tectal input on type X, XII and XIII depends to a large extent on the influence of type XIV neurons (see V.13).

2.3.6 The probability and influence of the assumptions made

The following basic assumptions underlie the preceding discussion:

(1) Tectal neurons can be classified in discrete cell types with specific dendritic and axonal properties.

(2) All neurons belonging to a certain, tectal cell type as distinguished in this thesis have a similar function.

(3) Within the tectum of the goldfish dendrites, dendritic shafts and cell bodies are postsynaptic structures; whereas axon terminals are presynaptic structures.

(4) All types of postsynaptic structures at a certain level have contacts with all types of presynaptic structures at the same level.

Assumptions (1), (2) and (3) have already been discussed when they were introduced (see section 2.3). The fourth assumption, however, requires some further discussion. This assumption means that the presynaptic elements present in a certain layer have no preference to make contacts with specific postsynaptic structures. This is of crucial importance for the schemes presented, since these would be almost meaningless when a large degree of specificity would appear to exist, e.g. when optic fibers would only terminate on certain cell types and would avoid others, or when, e.g., in layer 3/4 type I axons would only terminate on type IX neurons and telencephalic afferents only on type X neurons. Although such specificities in general cannot be excluded (cf. e.g. the specificity of the Purkinje cell-climbing fiber interaction in the cerebellum), they seem to be the exception rather than the rule, since a large number of examples of non-specific axonal termination patterns has been described for the central nervous system of vertebrates (see e.g. Guillery, '72; Raisman, '77; Somogyi, '78; White, '78; Peters et al., '79; Somogyi and Cowey, '81; Wilson et al., '81; Hornung and Garey, '81). Also for optic nerve terminals in the goldfish tectum no specificity has been observed in this respect (see chapter IVb, and e.g. Bunt et al., '78; Sharma, '81). Some evidence for the aspecificity of other presynaptic structures can be inferred from the similar mean size of contacts on all postsynaptic structures in a certain layer (see Discussion section of chapter IVb). So, in the tectum a refined stratification pattern seems to be used as a mechanism to realize specific patterns of neuronal connections rather than a high degree of specific cell to cell interactions. The latter would seem to make a refined stratification pattern redundant. So, assumption (4) seems rather plausible at present, but further investigations concerning this assumption are certainly necessary for definite insight in tectal circuitry.

Apart from the four basic assumptions enumerated above, the following three additional assumptions and/or simplifications were introduced to obtain an estimation of some quantitative aspects of tectal circuitry:

(5) All types of postsynaptic structures at a certain tectal level have equal percentages of contacts with all types of presynaptic structures present at the same level, except for the axons of type XIV.

(6) For type XIV neurons two situations were considered: a) type XIV axons exclusively make contacts with type XIV dendrites, b) axons of type XIV neurons make a large number of contacts on the other tectal cell types (see *APPENDIX* for precise percentages).

(7) The amount of influence of a certain type of presynaptic elements on a certain neuron is proportional to the number of synaptic contacts between both structures.

The following remarks should be made with respect to these quantitative assumptions.

Assumption (5) implies that all types of presynaptic elements present in a certain layer, except for the axons of type XIV, make equal numbers of contacts. This is certainly not always true, but too little information is available for more precise assumptions. To take layer 5 as an example: In this layer the axons of about 200.000 ganglion cells terminate, but only 2500-10.000 type III axons and 500-2000 type VII axons. The number of other types of presynaptic structures is unknown, as is the number of contacts made by the different types of presynaptic elements except for the optic nerve fibers (chapter IVb). For other layers a similar lack of information is encountered, which means that values dependent on this assumption do not reflect the real situation, but may only be used for mutual comparison. It should be noted, however, that assumption (5) is of rather low importance for the quantitative values calculated, since for the balance between the four main types of tectal input interactions *within* a layer are in general rather unimportant in comparison with interactions *between* layers. The latter depend on morphological characteristics of the tectal cell types, in particular on the location, dimensions and synaptic density of the dendrites, dendritic shafts and cell bodies. It has been shown in the present thesis that these characteristics are primarily determined by the tectal cell types and do not depend on influence from presynaptic elements (chapter III and IVb).

With respect to the different situations assumed for type XIV neurons (assumption 6a and b) the following should be noticed. The first assumption concerning type XIV, -type XIV axons would only make synaptic contacts with type XIV dendrites- is certainly too extreme, although there is evidence that type XIV neurons have at least some preference for making mutual synaptic contacts, since both their axons and dendrites have smaller synaptic contact zones than other tectal structures (chapter IVb). This extreme assumption, however, was only meant to analyse the rather strictly organized circuitry of type I-XIII neurons, which, in turn, could be used as a basis for an evaluation of the influence of the numerous and variable type XIV neurons on tectal circuitry. The opposite would be to assume no preference at all and to take the number of type XIV neurons as an estimate of their influence (see *APPENDIX*, condition 2) but this appears to be too extreme as well, since too less influence for other tectal neurons is left. Assumption 6b (see *APPENDIX*, condition 3), represents a more likely estimation with respect to the number of contacts of type XIV axons, but is still extreme in assuming no preference at all for type XIV neurons to make mutual synaptic contacts (see above). So, the reality is probably situated somewhere in between the results calculated for assumption 6a (condition 1 in the *APPENDIX*; fig. V.8-V.11, V.12a and V.13a) and those calculated for assumption 6b (condition 3 in the *APPENDIX*; fig. V.12b and V.13b).

Apart from the preceding remarks, it should be emphasized that type XIV neurons constitute a very heterogeneous population, of which only the average influence may be indicated. Various subpopulations, as shown in chapter III and depicted in figure 19 , page 51, might well have deviating connectivity patterns and consequently a different influence on tectal circuitry (see e.g. fig. V.11). At this moment, however, no information is available for a more detailed analysis of the functional significance of the different subpopulations of type XIV neurons in tectal circuitry.

The seventh and last assumption -the amount of influence of a certain presynaptic structure on a certain neuron is proportional to the number of synaptic contacts between both structures- is the most important one for quantitative estimations of tectal circuitry. It should be clear that "the amount of influence" is considered irrespective of the sign of this influence and that addition of several "amounts of influence" (see *APPENDIX*) has also been performed neglecting the sign of these influences, since these are unknown at present. Other physiological aspects of synaptology, such as spatial and temporal interactions and non-linearities are unknown as well, and are equally not incorporated in assumption (7). So, "*the amount of influence*" of a presynaptic structure on a postsynaptic structure is a value which is only related to the number of synaptic contacts; hence, this value is exclusively useful for mutual comparison of the involvement of different cell types in the processing of different inputs, and should not be confused with the *effect* of signals from a presynaptic structure on a postsynaptic structure, which can only be known from electrophysiological research.

Apart from the number of synapses, the distance of synaptic contacts to the origin of the axon is important as well for an estimation of the influence of a presynaptic structure on a cell type. Although precise knowledge concerning the relation between distance and influence is lacking for tectal neurons, it is well known that contacts close to the axon hillock of a neuron have a larger influence on the axonal signal of that neuron than synapses on distal parts of dendrites. This means that, in order to be true within a layer, assumption (7) and (5) together include the implicit assumption of a similar distribution of different types of synaptic contacts on each type of dendritic tree in a certain layer. This seems equally plausible as the lack of preference discussed for assumption (4). With respect to interactions between two or more dendritic trees of a cell type in different layers, the sites of origin of the axons tend to confirm the suggestions about the degree of involvement of the different cell types in the processing of different types of inputs (fig. V.13) rather than to enfeeble these suggestions. E.g., the origin of the axon of type XIII neurons in layer 2 stresses the importance of "deep" tectal input for this cell type, whereas the axon origin of type XII neurons in layer 4 is in agreement with the relatively larger importance of visual input for this cell type. Also, the site of origin of the axon of type IX and X (layer 3/4) stresses the importance of toral and telencephalic input for these cell types, whereas the relatively larger importance of visual input for type XIII is corroborated by the origin of the axon in layer 4/5. Also the importance of visual input for type XIV neurons is stressed by the site of the axon hillock, since this is located just at the point where the apical (visual signal processing) dendrites of these neurons converge their signals. Only for type I neurons speculations about the influence of the site of the axon hillock tend to weaken the large importance of toral input in favour of the visual input, since the visual signal processing dendrites in layer 5 are more close to the axon origin than the apical, toral signal processing dendrites in layer 7.

Finally it should be noted that the preceding discussion and schemes did not consider the possibilities of rather peculiar conditions, e.g. that there would exist specific synaptic configurations on the surface of dendritic shafts or around the axon hillock that could completely block signal propagation, or that the influence of synapses would strongly decrease with increasing distance to the axon hillock. When such conditions would appear to be the rule rather than the exception in tectal circuitry, the quantitative estimations would need a complete reevaluation. However, at present there are no indications for such peculiar conditions for the axo-dendritic and axo-somatic synaptic contacts in the tectum mesencephali of the goldfish, and a consideration of such conditions seems, consequently, not relevant for the present discussion. The only peculiar arrangement described until now is the dendro-axonal apposition in the arch of type $XIII_1$ neurons, but the functional significance of this arrangement is completely obscure at present.

2.3.7 Concluding remarks

The preceding discussion and the schemes presented are not meant as a precise description of tectal circuitry -for this too little information is available at present-, but only to construct a functional anatomical framework concerning the tectum mesencephali of the goldfish on the basis of present knowledge concerning its connections, cytoarchitecture and synaptology. The framework constructed allows for an analysis of the connectivity patterns of the tectal cell types and for an estimation of the relative degree of importance of these cell types in the processing of signals of different tectal afferent systems. The main conclusion is that presumably all tectal cells integrate different types of tectal input, which greatly stresses the multisensory integrative function of the tectum. For some cell types different types of input might even be of more or less equal importance (type VIII, IX, X, XII, XIII and the large type XIV neurons). For other cell types, however, one out of the different types of input is clearly dominant (for type I neurons: toral input; for type III, IV, VI, VII and the bulk of type XIV: visual input; for type XV: "deep" tectal input).

The framework constructed does not only allow for a tentative functional interpretation of the structure of the tectum. It is also useful for defining precisely where and how the frame should be reinforced, refined and filled in. Reinforcement of the frame has to be achieved by investigations in the field of the assumptions that have to be made, which means in particular in the field of the distribution of contacts with different types of presynaptic elements on the receptive surface of the cell types. Only when these distributions have been quantitatively analysed, a precise and well founded model of tectal circuitry can be achieved. Refining of the framework may be attained by further investigations on the lamination and termination pattern of the various types of tectal afferents and axons of tectal interneurons. At present, the information available for most tectal afferents is still insufficient for a detailed analysis of their influence and interactions in tectal circuitry. The numerous and heterogenous type XIV neurons should also be the object of more detailed observations. A definite filling in of the framework has to be achieved by electrophysiological studies. Morphological research, however precise it may be, only demarcates the potentialities of tectal circuitry. To what extent and in which way these potentialities are utilized under different conditions may only be understood from electrophysiological research. However, electrophysiological data that can be correlated with tectal morphology are hardly available at present (see the subsequent section). Only when more electrophysiological results will become available, a definite understanding of the functional organization of the tectum mesencephali of the goldfish and other teleosts comes into prospect.

2.4 Comparison with physiological data

2.4.1 Toral signal processing

Some electrophysiological aspects of the processing of toral input in the tectum have recently been investigated by Vanegas et al. ('79) in *Eugerres* and *Holocentrus* by means of electrical stimulation of the marginal tectal axons and subsequent extracellular recording of the electrical events throughout the depth of the tectum. Their results can be easily interpreted on the basis of the morphology of type I or pyramidal neurons, which are the only targets of marginal axons (Ito, '70; Laufer and Vanegas, '74a; Ito et al., '80; present thesis). The most important electrical events after stimulation of the marginal fibers are: 1) spike propagation along the marginal fibers with a conduction velocity of 0.20 or 0.16 m/sec; 2) monosynaptic depolarization of the apical dendrites of type I or pyramidal neurons; 3) an active current sink at the level where the apical dendrites of type I neurons converge to the dendritic shaft and 4) an activation of the axon terminals of type I neurons in layer 3/4 (Vanegas et al., '79). It is remarkable that this tectal afferent system, which receives its principal input from the cerebellum, has many physiological characteristics strongly reminiscent of that part of the brain, particularly of the parallel fiber-Purkinje cell system (Vanegas et al., '79).

The functional significance of the toral afferents to the tectum is obscure, since no further electrophysiological data are available. However, some speculations on the basis of comparative anatomical and behavioral data have been made. The occurrence of a valvula cerebelli, projecting to a torus longitudinalis which projects, in its turn, to type I tectal neurons via a peculiar marginal tectal layer, is unique for actinopterygians. The valvula cerebelli seems to some extent linked with the lateral line system, which may be mechanoreceptive as well as electroreceptive in teleosts. Both mechanoreceptors (Claas and Münz, '81) and electroreceptors (Finger et al., '81) may have topographically organized projections to the valvula cerebelli. Conceivably, the valvular-toral-tectal system might allow for correlation of the visually perceived environment and the environment as perceived by means of the lateral line system. Another, related function ascribed to the torus longitudinalis is opto-static correlation (Ariëns Kappers et al., '67; Ito, '71). Both functions are especially important for maintaining posture in water (Kishida, '79). It is noteworthy in this context that the torus longitudinalis and the stratum marginale of the tectum reach their highest degree of development in fishes that actively move from shallow to deep water or in turbulent water. In contrast, these structures are only poorly developed in epipelagic or bottom-dwelling fishes (Kishida, '79).

2.4.2 Visual signal processing

Electrical stimulation of the optic nerve has revealed that two retinal fiber populations with different conduction velocities terminate in the SFGS, both in *Eugerres plumieri* (Vanegas et al., '71, '74) and the goldfish (Schmidt, '79). A laminar profile analysis of postsynaptic potentials occurring after electrical stimulation of the optic nerve showed that the fastest group terminates more superficially than the more slowly conducting group. Histological verification of the recording site showed that their regions of termination roughly correspond to layer 5 and layer 4/5 respectively (Vanegas et al., '71; Schmidt, '79). Current theories indicate that the electrical potentials which can be recorded extracellularly in a laminated structure after massive electrical stimulation of a horizontally entering fiber system, exclusively represent the activity of long, vertically oriented dendrites or dendritic shafts (Nicholson and Freeman, '75; Freeman and Nicholson, '75; Schmidt, '79). The activity of horizontally oriented dendrites, which are abundant in layer 4/5 and 5, is not represented in these potentials. Consequently, only type I, XII and XIV are considered to be responsible for the postsynaptic phenomena recorded (Vanegas et al., '74; Schmidt, '79). All three of these types of neurons indeed have been shown to make contacts with optic nerve fibers (present thesis, chapter IV). Type I neurons are most probably contacted by the slowly conducting fibers in layer 4/5, as could be concluded from the depth of recordings of postsynaptic action potentials (Vanegas et al., '74) or the distribution of electrical sources and sinks through the tectal layers (Schmidt, '79). Type XII and type XIV neurons may be contacted by both "slow" and "fast" conducting retinal fibers. A tentative scheme correlating the electrical events recorded with the morphology of type XII and/or type XIV neurons is proposed by Vanegas ('75). The retinal fibers that terminate in layer 2 have a slower conduction velocity than both populations of fibers terminating in layer 5 and 4/5 (Schmidt, '79). The results of Schmidt ('79) did not allow for conclusions about cell types that are postsynaptic to this third population of retinal efferents.

A number of papers deal with responses of single tectal units after visual stimulation of the contralateral eye. For a correct interpretation of the results obtained it is necessary to know whether the responses represent the activity of retinal fiber terminals or the activity of intrinsic tectal neurons. A definite elucidation was recently achieved by Rowe ('80) and Niida et al. ('81) by means of intracellular recording and subsequent dye injection. Most investigations, however, used extracellular recording, for which more indirect ways of discrimination have to be applied.

Reliable criteria to distinguish between the activity of retinal fiber terminals and tectal neurons were established by Sutterlin and Prosser ('70) and O'Benar ('76), using electrical stimulation of the optic nerve and subsequent measurement of the latency of the response. Units firing after a latency period smaller than 2 msec are in general retinal fibers, whereas tectal units mostly fire after a latency of 3 to 10 msec (Sutterlin and Prosser ,'70; Vanegas et al., '71, '74; O'Benar, '76; Riemslag and Schellart, '78; Niida et al., '81). In addition, the spike responses of tectal units and retinal fibers upon electrical stimulation of the optic nerve differ with respect to (1) the number of spikes elicited, (2) habituation, (3) maximal frequency of firing and (4) the shape, amplitude and time course of the action potentials. In particular the last aspect was used by Guthrie and Banks ('74, '76, '78) for identifying the responses of intrinsic tectal units. Galand and Liege ('75) used the depth of recording as a criterion. Units in layers 3 and 4 were considered as tectal neurons, since no retinal fibers occur in this layer. However, units in layer 5 may be both retinal fibers and tectal neurons, when no further criteria are available (Galand and Liege, '75; Schellart and Spekreyse, '76; Riemslag and Schellart, '78; Schellart et al., '78). Jacobson and Gaze ('64), Cronly-Dillon ('64), Zenkin and Pigarev ('69), Niida and Sato ('72b) Ramstad and Hughes ('73) and Warzok and Marks ('73), all made recordings of tectal units without identifying these units. However, judging from the location of the recording site in the SFGS and the use of metal electrodes, their results most likely represent optic fiber responses. Metal electrodes appear very selective in recording the activity of retinal fiber activity (Sutterlin and Prosser, '70; O'Benar, '76), whereas micropipettes, filled with a saline-solution, may record the activity of both fiber terminals and neurons, with preference for the latter (Sutterlin and Prosser, '70; O'Benar, '76).

Retinal fibers that terminate in the tectum show a number of different types of responses after visual stimulation of the contralateral eye (e.g. sustained as well as transient on, off and on-off responses; movement and direction selectivity; Jacobson and Gaze, '64; Cronly-Dillon, '64; Sutterlin and Prosser, '70; Niida and Sato, '72; Ramstad and Hughes, '73; Warzok and Marks, '73; Galand and Liege, '75; O'Benar, '76). However, these types of responses in general do not show any specific stratification pattern, nor any specific topographical distribution. Only one type of stratification pattern has been mentioned, viz. by Jacobson and Gaze ('64), who noticed that in layer 4/5 of the goldfish tectum only sustained responses occur. This was confirmed by Cronly-Dillon ('64), Warzok and Marks ('73) and O'Benar ('76), but was not found in other teleosts (Niida and Sato, '72; Ramstad and Hughes, '73). It is obscure whether these sustained responses may be ascribed to the slowly conducting population of fibers which also occurs in layer 4/5 (Schmidt, '79).

Extracellular recordings of tectal units allow for a distinction of three populations of visual tectal neurons. One population is located in layer 5 and 4/5, the SFGS, a second one in layer 4 and 3 and a third one in layer 1.

Sutterlin and Prosser ('70) and O'Benar ('76) found in the SGFS a particular population of neurons showing spontaneous firing in the dark, inhibited by light as well as by electrical stimulation of the optic nerve (type I of Sutterlin and Prosser, '70 and the simple, non-habituating cells of O'Benar, '76). The tectal neurons recorded by Schellart et al. ('78) also occur in the SFGS, but these showed partly different characteristics. The neuronal population described by Guthrie and Banks ('74, '76, '78) was not localized in any specific layer, but includes neurons in the SFGS as well. Several morphologically distinct cell types have been described in the SFGS (type I, III, IV, VI and VII), and all may be responsible for the responses observed. In addition, terminals from cells in the nucleus pretectalis, area pretectalis and nucleus isthmi terminate in the SFGS, and these might also be considered as candidates for some of the responses recorded in the SFGS. Their visual responses would at least have long latencies and other characteristics related to trans-synaptical stimulation.

Neuronal responses in layers 4 and 3 (the SGC) and layer 2 (the SAC) distinguish themselves by a number of peculiar characteristics. These may include the occurrence of rhythmic spontaneous activity (Sutterlin and Prosser, '70; Guthrie and Banks, '74), habituation (newness-neurones; Galand and Liege, '75; O'Benar, '76; Guthrie and Banks, '78); large receptive fields and binocularity (Guthrie and Banks, '74; Galand and Liege, '75) and a rather large "plasticity" in response (O'Benar, '76). In addition, multimodality is encountered in the SGC and SAC (see below). There are no indications regarding specific cell types which would be responsible for these responses. Consequently, all neurons occurring in the SGC or SAC might be responsible for the types of responses encountered, since all receive monosynaptic and/or polysynaptic input from retinal fibers (see above; type V, VIII, IX, X, XI, XII and XIII). Guthrie and Banks ('78) distinguished eight types of responses, based on specific visual stimulation conditions. These could be recorded from layer 1 up to layer 5, with a slight preference for layer 4/5, 3/4 and 1. This might be correlated with the larger density of cell bodies in these layers, but does not allow for identification of specific cell types. In contrast, it might even suggest that the response types distinguished are randomly distributed over the different morphologically distinguishable cell types.

Electrical activity of periventricular neurons could be recorded satisfactory only by O'Benar ('76) in the goldfish. Recordings from layer 1 revealed multi-unit burst-like activity of simultaneous firing neurons suggesting electric coupling. This might be correlated with the gap-like junctions described in the present thesis. In most recordings, the activity could be changed by visual stimuli, which is in keeping with the occurrence of synapses with retinal fibers on the dendrites of these neurons (present thesis chapter IV).Visual stimulation could elicit a large variety of response-types, sometimes rather irreproducable or "plastic" (O'Benar, '76). Guthrie and Banks ('78), investigating tectal neurons in the perch, also included SPV cells in their results, but could not distinguish the activity of SPV neurons from that of other tectal neurons.

Because the axon hillock of the periventricular or type XIV neurons is situated far away from the cell body, viz. in layer 4/5, the functional significance of spike recordings in layer 1 is unclear. In the frog, the same problem is encountered. In this animal, Gruberg and Lettvin ('80) showed that the cell bodies of periventricular neurons revealed no or very erratic electrical activity. However, in the nucleus isthmi, a cell mass getting its input in the frog exclusively from periventricular tectal cells (Gruberg and Udin, '78; Gruberg and Lettvin, '80) good and well-defined responses could be recorded. Gruberg and Lettvin ('80) conclude that the somata of periventricular cells have no essential function in the electrical activity of these neurons. A similar situation might well be present in teleosts. Also in teleosts, SPV- or type XIV neurons are the most important afferent elements for the nucleus isthmi (Ito et al., '81b) and in the nucleus isthmi well defined electrical activity can be recorded after stimulation of the optic nerve (Williams and Vanegas, '81). So, responses recorded in layer 1 seem to represent only residual electrical activity coming from the more superficially located axon hillock, rather than the full electrical activity of these neurons.

The intracellular recordings of Rowe ('80) in *Ambloplites rupestris* revealed a type of visual tectal neuron with a cell body in the boundary region between layer 7 and 6 and dendrites in layer 5 or below. These neurons might well correspond to type III neurons in the goldfish, since these neurons have some deeper located branches in teleosts with a highly developed visual system, to which *Ambloplites* belongs (Kishida, '79). Type III neurons indeed receive monosynaptic retinal input (chapter IV) and represent a visual type of tectal interneuron (see fig. V.13). The neurons identified by Rowe ('80) showed a quite characteristic type of response, (on-off burst cells), but were not selective for specific stimulus conditions.

The intracellular recordings of Niida et al. ('81) in *Carassius carassius*, a species closely related to *Carassius auratus*, identified visual neurons in layer 1 (type XIV), in layer 3/4 (which might be type IX or type X neurons), in layer 4/5 (which might be type IV or type VII neurons) and in layer 5 (type I neurons). This is in accordance with the present morphological study and with previous extracellular recordings. By far most recordings were obtained from type I neurons, which is probably caused by the large frequency of occurrence of these neurons (chapter III) and by their good resistance to electrode penetration due to their large dimensions in the direction of electrode penetration. Type I neurons showed a number of different types of responses, corresponding to the responses recorded extracellularly in layer 5 (Sutterlin and Prosser, '70; Guthrie and Banks, '74; O'Benar, '76; Schellart et al., '78). The most remarkable result of Niida et al. ('81) is the complete absence of any correlation between the type of response recorded and the cell type identified. Type I neurons showed a number of different types of responses, whereas other types of neurons showed the same type of response as some type I neurons. A similar lack of correlation was reported by Guthrie and Banks ('78). In my opinion, the "randomness" of these effects indicates that the aspects of visual responses studied (on-off responses, transient and sustained responses) do not encode relevant information for the goldfish. The tectum mesencephali of teleosts apparently is no analyser of on-off or transient-sustained visual responses, but of other aspects of visual as well as non-visual information. This is in agreement with the conclusion of the previous section, that tectal cytoarchitecture suggests an important multimodal integrative function.

The pronounced responses of type I neurons upon visual stimulation (Niida et al., '81) might at first sight seem somewhat surprising, because they have only a low percentage of contacts with retinal fibers (chapter IV; see also fig. V.13). However, these contacts are closer to the axon hillock than those with toral afferents, which might enhance the influence of visual stimulation. However, purely visual stimuli are rather unphysiological, and the experiments of Niida et al. ('81) do not indicate the balance between toral and visual stimulation. When both toral and visual input would be provided to type I neurons in a physiological ratio, it might well be possible that the visual input would appear to be of relatively low importance. It should be mentioned that the visual input of type I neurons in the goldfish seems to be smaller than in *Eugerres*. In the goldfish, contacts with retinal fibers occur almost exclusively on their dendrites in layer 5 (chapter IV), whereas in *Eugerres* a substantial number of contacts occur on the dendritic shaft as well (Laufer and Vanegas, '74a). According to Ebbesson ('80), in the visually highly developed teleost *Holocentrus*, type I neurons would receive no retinal input at all, which would be a very remarkable species difference. However, the statement of Ebbesson ('80) can only be evaluated after publication of more experimental details.

2.4.3 Deep tectal information processing - multimodality

With respect to non-visual responses in the tectum of teleosts, only a few rather incidental observations are available. Sutterlin and Prosser ('70) and Guthrie and Banks ('74) have reported spontaneously firing neurons in deep tectal layers that do not respond to visual stimulation. Galand and Liege ('75) described three types of multimodal units for deep tectal layers: visuo-tactile units; visuo-lateral line units and visuo-acoustic units. Visuo-tactile units in deep tectal layers have also been described by O'Benar ('76). Visuo-lateral line responses and visuo-acoustic units have also been described by Callens et al. ('67) and Niida ('73) respectively, however, without reference to a particular tectal layer. Guthrie and Banks ('74) recorded the activity of deep tectal neurons during electrical stimulation of the fasciculus longitudinalis lateralis in the rhombencephalon. This type of stimulation could have both excitatory and inhibitory effects on deep tectal neurons. So, several types of multimodal responses have been demonstrated in the tectum, and these predominantly or possibly even exclusively occur in deep tectal layers, where afferents from the contralateral tectum, the torus semicircularis and the reticular formation have been demonstrated. There are no physiological indications about the tectal cell types which may be responsible for the multimodal responses, but from the present study type XII and XIII are the most likely candidates. This agrees with the location of multimodal units in the SAC and SGC.

To my knowledge, there are no data available concerning the electrophysiological properties of telencephalic afferents to the tectum.

3 *TECTAL EFFERENTS*

3.1 *Tectal targets*

The efferents of the goldfish tectum have recently been investigated by Grover and Sharma ('79). Their results, combined with the results of Schmidt ('79) concerning tecto-retinal efferents in the goldfish, are summarized in figure V.14. The nuclei mentioned in this figure are indicated in figure V.3, V.4 and V.5. The nucleus isthmi, not identified as a tectal target in the goldfish by Grover and Sharma ('79), is included in figure V.14 for reasons to be discussed below. Tectal efferents have also been investigated in the teleosts *Eugerres* and *Holocentrus* (Ebbesson and Vanegas, '76), the blind fish *Astyanax hubbsi* (Sligar and Voneida, '76), and the carp *Cyprinus carpio* (Luiten, '81). These studies agree with the results of Grover and Sharma ('81) in describing ascending, bilateral projection to the nucleus rotundus and other pretectal cell groups, a medial projection to the contralateral tectal half and descending projections to the ipsilateral torus semicircularis, other ipsilateral dorsolateral tegmental areas, the ipsilateral lateral reticular formation and the contralateral medial reticular formation (see fig. V.14). The descending efferents constitute the largest efferent tectal tract (tractus-tecto-bulbaris). Ebbesson and Vanegas ('76) and Luiten ('81) describe an additional medial projection to the torus longitudinalis. However, the toral afferents labelled represent most likely the cerebello-toral afferents, which traverse the tectum before reaching the torus (Ito and Kishida, '78).

The most important difference between the results of Grover and Sharma ('79) and those of other authors mentioned concerns the tecto-isthmic projection, which has not been described by Grover and Sharma ('79), but was demonstrated in all other teleosts investigated. Moreover, Ito et al. ('81) and Sakamoto et al. ('81) demonstrated in the teleost *Navodon modestus* that the tecto-isthmic projection is highly ordered, and Williams and Vanegas ('81) provided electrophysiological evidence for the existence of a tecto-isthmic

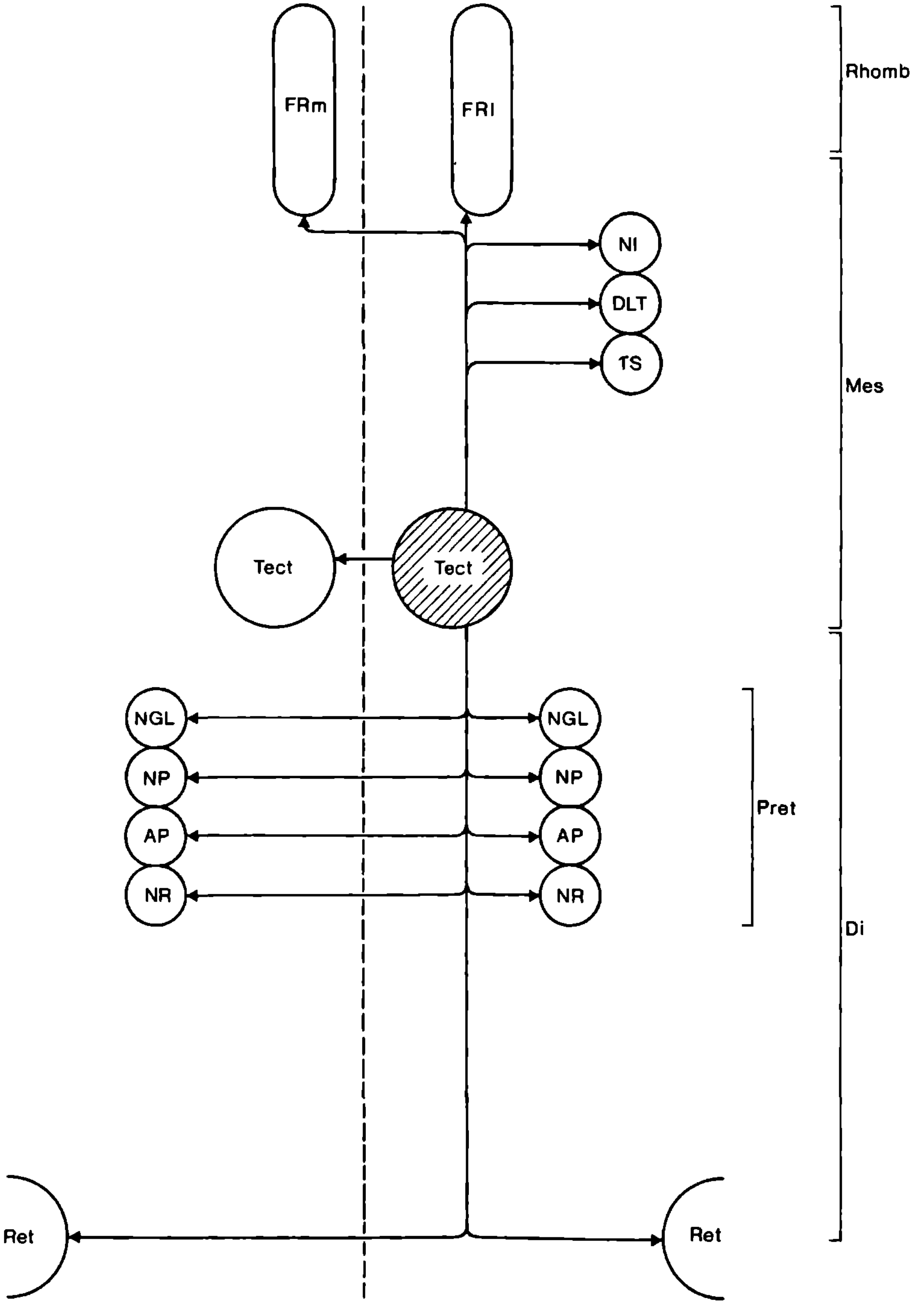


Fig. V. 14. Schematic representation of present knowledge concerning the efferent connections of the tectum of the goldfish. For references, see text, section 3.1. For abbreviations, see pag. 110.

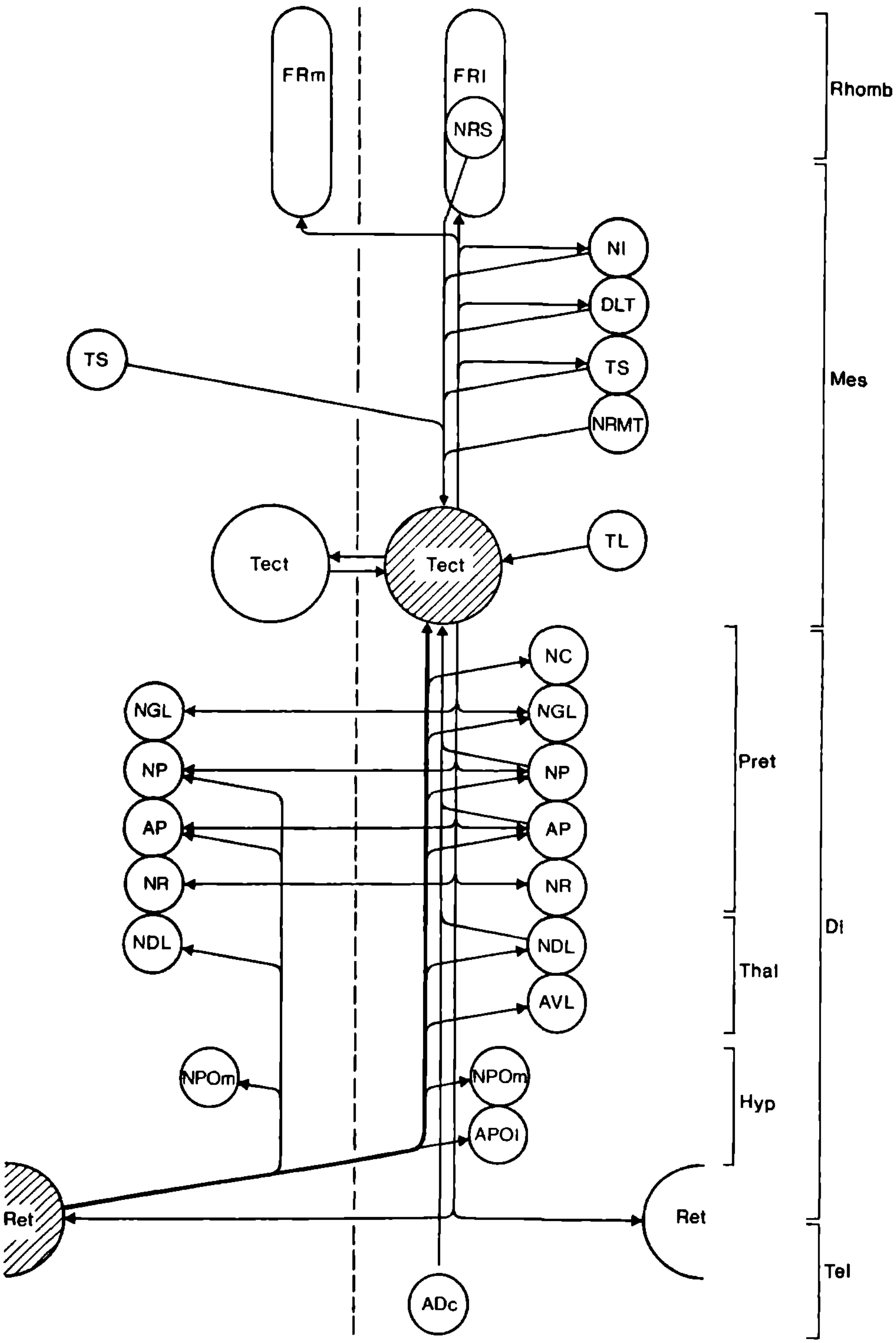


Fig. V. 15. Schematic representation of present knowledge concerning the extrinsic connections of the retina and the tectum in the goldfish. For references: see text, sections 1.1, 1.2 and 3.1. For abbreviations, see pag. 110.

projection in *Holocentrus* and *Eugerres*. In fact, in all classes of vertebrates investigated, the nucleus isthmi or its homologue, the nucleus parabigeminalis (Gruberg and Udin, '78; Sakamoto et al., '81) receives the bulk of its input from the tectum mesencephali (teleosts: Ito et al., '81; Sakamoto et al., '81; amphibians: Robinson, '68; Wilczynski and Northcutt, '77; Gruberg and Udin, '78; Gruberg and Lettvin, '80; reptiles : Foster and Hall, '75; birds: Hunt and Künzle, '76, '77; mammals: Graham, '77; Harting, '77; Baleydier and Magnin, '78; Méndez-Otero et al., '80) and is considered as a "satelite system" of the tectum or colliculus superior (Graybiel, '78). So, a nucleus isthmi without tectal input would be quite unlikely. Most probably, the deviating results of Grover and Sharma ('79) with respect to the tecto-isthmic projection will have some technical reason rather than to reflect species differences. Consequently, also in the goldfish the nucleus isthmi should be considered as a tectal target (fig. V.14), just as in the other teleosts investigated.

The existence of a tecto-retinal projection is a point of controversy in the literature. Anatomical (Witkovsky, '71; Stell, '72) and electrophysiological studies (Vanegas et al., '73; Sandeman and Rosenthal, '74; Schmidt, '79) have clearly demonstrated the existence of retinopetal, centrifugal fibers in teleosts. However, the origin of these fibers is not certain. Electrophysiological results strongly suggest a tectal origin of retinopetal fibers. Using electrical stimulation of the optic nerve, Vanegas et al. ('73) found in *Eugerres* a fast-conducting population of fibers that showed several characteristic of antidromic activation. Moreover, these fibers remained intact after eye-enucleation, which excludes a retinal origin and suggest a tectal origin. Similar electrophysiological results were obtained by Schmidt ('79) in the goldfish. Electron-microscopical studies corroborated the persistence of large caliber (=fast conducting) fibers after eye-enucleation (Laufer and Vanegas, '74b).Sandeman and Rosenthal ('74) recorded the activity of retinopetal fibers in the trigger fish *Hemibalistes chrysopterus*, which could be activated by visual, vestibular, vibratory and tactile stimuli. Their responses were weakened by ablation of the ipsilateral tectum and abolished by ablation of the contralateral tectum, strongly suggesting a tectal origin.

Anatomical studies, using HRP injections in the eye or optic nerve, have yielded less consistent results. Schmidt ('79) has reported for the goldfish the bilateral labeling of a large number (about 1500) of vertically oriented bipolar tectal neurons in the SFGS. This labeling could only be obtained when the eye had been enucleated 2 to 6 weeks before. Some of the neurons shown by Schmidt ('79) probably represent type I neurons, because of the characteristic bifurcation of the apical dendritic shaft at the boundary of layer 6 and 7 or because of the basal dendritic shaft in layer 4. Type I neurons, however, are clearly interneurons, not projecting outside the tectum (present thesis). Other neurons labeled by Schmidt ('79) might represent type VI neurons, which indeed have efferent axons. However, these axons are not of a large caliber and do not course through the SO or SFGS as could be expected from the electrophysiological results (see above). Meyer and Ebbesson ('81) and Meyer et al. ('81) also labeled tectal neurons after HRP injection in the eyes of *Synodontis nigriventris* and *Tetradon fluviatilis*, respectively. However, these represent other cell types than those labeled by Schmidt ('79), because they are located both in the SFGS and SGC, while those in the SFGS are not bipolar. Furthermore, the number of neurons labeled by Meyer and Ebbesson ('81) and Meyer et al. ('81) is considerably smaller than the number of neurons labelled by Schmidt ('79). Apart from the tectal neurons, Meyer and Ebbesson ('81) and Meyer et al. ('81) labeled neurons in the pretectum and dorsolateral thalamus. These were not labeled by Schmidt ('79). Münz and Claas ('81), using poecilid and cyprinid fishes, labeled neurons in the pretectal region as well, but did not find labeling in the tectum. They also found telencephalic neurons projecting to the retina. Peyrichoux et al. ('77), using cyprinid fishes, could not at all produce reproducable labeling of retinopetal cells.

Apart from species differences, which I consider unlikely for all differences mentioned, a number of technical reasons may be involved. Absence of labeling may be due to the inability of retinopetal fiber terminals to incorporate HRP (Peyrichoux et al., '77). On the other hand, false labeling may occur. According to Münz (personal communication) intraocular injection of HRP results in aspecific , weak labeling of tectal neurons after three days survival time, caused by degeneration of anterogradely filled optic nerve fibers and subsequent incorporation of the HRP delivered in tectal neurons. Such a kind of labeling of tectal neurons might be present in the material of Meyer and Ebbesson ('81), who show only weak labeling of tectal neurons after long survival times. The massive labeling of bipolar neurons in the SFGS described by Schmidt ('79), might also be due to local uptake of HRP by the neurons labeled. In experiments of this type, HRP might reach the tectum by means of capillary transport through the empty channels in the optic nerve. There seems to be no other explanation for the dramatic effect of eye enucleation and the labeling of interneurons (type I). Nevertheless, the results of Schmidt ('79) are still incorporated in figure V.14, since at present the existence of tecto-retinal fibers cannot be excluded.

It should be mentioned that a number of observations may be explained by assuming that pretectal neurons have an axon terminating in the retina and a collateral terminating in the tectum (Münz, personal communication). This would be in accordance with the labeling of pretectal neurons after HRP injection both in the retina (Meyer and Ebbesson, '81; Meyer et al., '81; Münz and Claas, '81) and the tectum (Grover and Sharma, '81; Luiten, '81), with the continued existence of fast conducting fibers in the tectum after eye enucleation (Vanegas et al., '73; Schmidt, '79) and with their large caliber and superficial course in the tectum (Laufer and Vanegas, '74b). Such a large caliber and superficial course was never observed for efferent tectal axons (present thesis), whereas pretectal neurons may have very large axons (Ito et al., '81) and project to superficial tectal layers (Grover and Sharma, '81). The postsynaptic tectal evoked potential recorded by Schmidt ('79) after eye enucleation might be explained as well by the existence of pretectal neurons projecting to both the retina and the tectum, and the results of Sandeman and Rosenthal ('74) would be explainable by assuming that they did not only ablated the tectum, but also the pretectum. The antidromic spike described by Vanegas et al. ('73) is more difficult to explain. However, pretectal cells with collaterals to both the retina and the tectum do not exclude the concurrent existence of tectal cells that project to the retina.

As far as I am aware only two electrophysiological studies deal with tectal efferents. Mark and Davidson ('66) recorded single unit activity in the intertectal commissure of *Astronoatus ocellatus*. These units showed rhytmic spontaneous activity in the dark, which was mostly inhibited by increasing levels of background illumination, but could not be changedby patterned visual input, such as light or dark objects or moving stimuli. Williams and Vanegas ('81) recorded electrical activity in the nucleus isthmi, nucleus rotundus, nucleus dorsolateralis tegmenti and corpus glomerulosum after electrical stimulation of the tectum or the optic nerve in *Eugerres* and *Holocentrus*. Neurons in the nucleus isthmi showed a characteristic burst of spikes after tectal stimulation. The results further suggest that the tectal neurons projecting to the nucleus isthmi receive monosynaptic input from retinal fibers. The responses in the nucleus rotundus (or nucleus prethalamicus) showed two distinct excitatory components. The results concerning the nucleus dorsolateralis tegmenti and corpus glomerulosum were only fragmentary.

Electrical stimulation of the tectum of teleosts elicits several types of conjugate eye and body movements (Chauchard and Chauchard, '27a, b; Akert, '49; Meyer et al., '70; Demski and Gerald, '74). The direction of these movements depends on the tectal site which is stimulated, suggesting a kind of motor-map within the tectum (Akert, '49; Meyer et al., '70). These behavioral responses should be effectuated via tectal efferents. Eye movements elicited by tectal stimulation may be mediated by the nucleus pretectalis, which receives bilateral tectal input (see fig. V.14) and projects to the oculomotor nuclei (Finger and Karten, '78). Body movements may be mediated by the reticular formation, which is an important target of tectal efferents and projects to the spinal cord. The nucleus pretectalis, area pretectalis and the nucleus isthmi may be involved as well, since these tectal targets project to the cerebellum (Finger and Karten, '78; Grover and Sharma, '81; Luiten, '81).

Figure V.15 summarizes the present knowledge concerning the extrinsic connections of the tectum and retina of the goldfish. Eight structures appear to have reciprocal connections with the tectum (retina; area pretectalis; nucleus pretectalis; contralateral tectal half; torus semicircularis; nucleus dorsolateralis tegmenti; nucleus isthmi; and the ipsilateral reticular formation, in particular the nucleus reticularis superior). Four nuclei project to the tectum without receiving tectal input (the telencephalic area dorsalis centralis; nucleus dorsalis lateralis thalami; torus longitudinalis and the nucleus of the rostral mesencephalic tegmentum or nucleus ruber), whereas three structures receive tectal input without projecting back to the tectum (nucleus rotundus; nucleus geniculatus lateralis; contralateral reticular formation). The nucleus dorsalis lateralis thalami, the area pretectalis and the nucleus pretectalis are intercalated in three indirect retinotectal pathways. So, the pattern of extrinsic tectal connections is rather complex, suggesting an important integrative function.

3.2 Efferent tectal neurons

The Golgi-study presented in chapter III revealed that four types of tectal neurons have myelinated axons leaving the tectum (type VI, X, XII and XIII). Electron microscopical observations (chapter IV) confirmed this finding for type VI, XII and XIII neurons. Type X has not yet been investigated electron-microscopically. Recent investigations of Grover and Sharma ('81) corroborated the efferent character of type XII and type XIII neurons, since both types of neurons were labelled after HRP injection in the torus semicircularis or in the tractus tecto-bulbaris. According to the results of Schmidt ('79), extensively discussed in the preceding paragraphs, the axons of type VI neurons might terminate in the retina. HRP injection in tectal targets did not yet label neurons that could belong to type X.

The present study revealed that type XIV neurons have unmyelinated axons with abundant collateral terminations within the tectum (chapters III and IV). We never could observe a collateral leaving the tectum or coursing along a substantial distance in layer 2. However, recent investigations using HRP injection in tectal targets revealed at least three sites of termination of type XIV axons outside the tectum, i.e. the nucleus isthmi, the pretectum and the contralateral half. This means that some collaterals of type XIV axons still do leave the tectum. Ito et al. ('81) labeled a peculiar subtype of type XIV neurons after HRP injection in the nucleus isthmi of *Navodon modestus*. This subtype, with a dendritic as well as an axonal arborisation in layer 6, has also been demonstrated in the goldfish (present study, chapter III, p.43; see also cell a in fig. 16, p.42 and fig. 19,p.51). Conceivably, also in the goldfish the basal axon collateral of this subtype projects to the nucleus isthmi.

Grover and Sharma ('81) demonstrated in the goldfish two other targets of type XIV axons, viz. the pretectum and the contralateral tectal half. HRP injection in the pretectum, including the area pretectalis and nucleus rotundus, but not the nucleus pretectalis and nucleus geniculatus lateralis, labeled monopolar neurons in the tectal layers 1 or 2, with an apical process reaching layer 5, thus allowing for identification as type XIV neurons. Retrograde labeling in the contralateral tectum was difficult to obtain, but is one out of 15 attempts, Grover and Sharma ('81) clearly labeled neurons

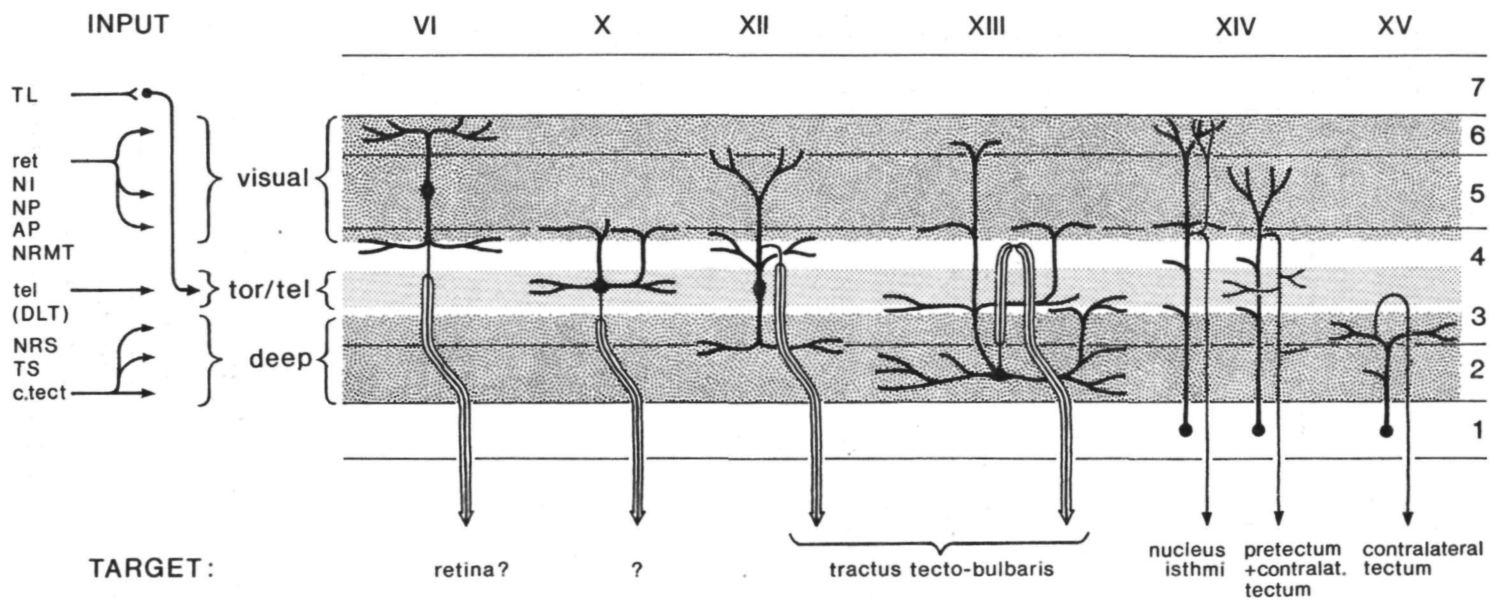


Fig. V. 16. Summarizing representation of present knowledge concerning the efferent tectal neurons in the goldfish. For references, see text, section 3.2. The afferent tectal zones indicated are discussed in section 2.2. For abbreviations, see pag 110.

in the SPV. Although some of the labeled cells had a peculiar multipolar shape, most of them might represent type XIV or type XV neurons. A projection of type XIV neurons to the contralateral tectal half is supported by two other findings. At first, O'Benar ('76) recorded in the SPV electrical activity closely resembling the activity of intertectal commissural fibers as recorded by Mark and Davidson ('66). Secondly, Ito et al. ('81) describe degeneration of some axon terminals of the S5 type in the tectum of *Holocentrus* after lesions in the contralateral tectal half. As is discussed in chapter IVb, S5 terminals closely resemble the axon terminals of type XIV axons as identified in the goldfish (present thesis).

The present knowledge concerning the projections of efferent tectal neurons is summarized in figure V.16. Type VI neurons, receiving predominantly visual input (see fig. V.13 and the general discussion section 2.3.2), might project back to the retina. The target of the axons of type X neurons, which integrate visual, toral and telencephalic input, is unknown. The axons of type XII and XIII neurons, which integrate all types of tectal input, constitute the tractus tecto-bulbaris, which is the most important efferent tract of the tectum. Type XIV neurons, which also may integrate all types of tectal input, however, with visual input as dominant modality, may project to the area pretectalis, the nucleus pretectalis, the contralateral tectal half and the nucleus isthmi. Type XV neurons, processing predominantly non-visual, deep tectal input, might also project to the contralateral tectal half. Thusfar the tectal neurons projecting to the nucleus pretectalis, nucleus geniculatus lateralis and nucleus dorsolateralis thalami have not yet been identified.

4 CONCLUSIONS

The preceding discussion is aimed at an exploration of the functional significance of the laminar organization of the tectum at both the cellular and the synaptic level, It has been pointed out that the tectum mesencephali of the goldfish does not only receive afferents from the retina, but also from at least ten other brain centers. Each type of tectal afferent has a characteristic level of termination within the tectum, allowing for a distinction of four main afferent zones: a toral afferent zone (layer 7), a visual afferent zone (layers 5/6, 5 and 4/5), a toral/telencephalic afferent zone (layer 3/4) and a deep afferent zone (layers 3 and 2), with both visual and non-visual afferents. The tectal cell types differ primarily with respect to the location and extension of their dendrites in one or more of these distinct afferent zones, suggesting that each cell type receives a characteristic sample out of the information available from different sources. The efferent neurons that give rise to the large tractus tecto-bulbaris, receive input from all afferent tectal zones thus probably integrating all types of tectal input. It may be concluded that the laminar organization of the tectum is primarily relevant for multimodal integration.

The tentative conclusion formulated above has been compared with electrophysiological data. In general, these do not present evidence for special functions of tectal neurons in visual discrimination, since (1) many neurons do not respond at all to the visual stimuli used, (2) visual responses of tectal neurons are in general not more complex than those of retinal ganglion cells, and (3) the types of responses recorded seem to be randomly distributed among the different cell types. Tectal neurons differ from retinal ganglion cells only in their strong habituation to visual stimuli and in their responses to non-visual stimulation. This is in accordance with the suggestion that tectal neurons have a multimodal integrative function rather than a visual discrimination function. However, this suggestion can not yet be substantiated, since systematical electrophysiological investigations concerning multimodal integration within the tectum have not yet been reported.

Apart from its refined laminar organization, the tectum mesencephali has also a detailed topographical organization of both its afferent and efferent connections. The combination of a laminar and topographical organization of tectal afferents is particularly suited for the localization of objects in the environment of the animal on the basis of integrated multimodal information (including visual, acoustical, lateral line and tactile afferent input). The topographical organization of tectal efferents allows for subsequent orientation of the eyes, head and/or body towards or away from relevant objects. This has particularly become clear from focal electrical stimulation of the tectum, which elicits goal directed movements, the direction of which depends on the tectal site stimulated.

The general function of the tectum, viz. localization and selection of stimuli and generation of orientation movements, has remained rather constant throughout all levels of vertebrate phylogeny (Ingle and Sprague, '75). However, the following two peculiarities of the teleostean tectum in comparison with the tectum of terrestral vertebrates can be noticed. The first one is the occurrence of a marginal layer, possibly related with the lateral line system, a pressoreceptive sensory system occurring only in aquatic vertebrates. The second one is the importance of multimodal integration as suggested by the present analysis of tectal cytoarchitecture and synaptology and supported by the low efficacy of exclusively visual stimulation in evoking spike responses of tectal neurons. This might suggest that, whereas in terrestral vertebrates different types of stimuli each may separately result in efferent tectal activity (see Ingle and Sprague, '75 for review), in the aquatic teleosts the simultaneous presence of stimuli of different modality is an important prerequisite for activity of efferent tectal neurons. Conceivably, in an aquatic environment exclusively visual stimuli, not attended with acoustical, lateral-line or tactile signals, might more readily represent non significant reflections than in a terrestral environment.

To arrive at a quantitative estimation of the degree of influence of the main types of tectal input on the various cell types distinguished, several calculations have been performed based on the following assumptions and/or simplifications (see also section 2.3.6 of the general discussion):

(1) Tectal neurons can be classified in discrete cell types with specific dendritic and axonal properties.

(2) All neurons belonging to a certain tectal cell type as distinguished in this thesis have a similar function.

(3) Within the tectum of the goldfish, dendrites, dendritic shafts and cell bodies are postsynaptic structures, whereas axon terminals are presynaptic structures.

(4) All types of postsynaptic structures at a certain level have contacts with all types of presynaptic structures at the same level.

(5) All types of postsynaptic structures at a certain level have equal percentages of contacts with all types of presynaptic structures present at the same level, except for the axons of type XIV neurons.

(6) a: type XIV axons make exclusively synapses with type XIV dendrites

b: type XIV axons make a large number of synapses with dendrites of other types of tectal neurons.

(7) The influence of a certain axon on a certain neuron is proportional to the number of synaptic contacts between both structures.

All calculations start from the assumptions 1, 2, 3, 4 and 7. However, with respect to the assumptions 5 and 6 the following three conditions are considered.

Condition 1 starts from the assumptions 5 and 6a. This means that any type of post-synaptic structure at a certain level makes equal percentages of contacts with all types of presynaptic structures present at the same level, except for the axons of type XIV neurons, which are presumed to make only synaptic contacts with dendrites of type XIV neurons.

Condition 2 implies that the assumptions 5 and 6 are replaced by the assumption that each individual axon makes about the same number of synaptic contacts, which means that the influence of each type of axon is proportional to its frequency of occurrence.

Condtion 3 starts from the assumptions 5 and 6b, which implies, just as in condition 1, that any type of postsynaptic structure at a certain level makes equal percentages of contacts with all types of presynaptic structures present at the same level, except for the axons of type XIV neurons. In contrast to condition 1, type XIV axons are now assumed to establish a large percentage of contacts with the other tectal neurons.

The following symbols are used: i (cell type) means the input of a cell type (eg i (III), i (IV) etc); o (cell type) means the output of a cell type; the four main steams of tectal afferent input are indicated as vis, tel, tor or deep, which means visual, telencephalic, toral and "deep" tectal input, respectively. For a further discussion of these types of input the reader is referred to the general discussion.

Condition 1

For tectal interneurons the following equations can be formed.

Type I: is contacted by all tectal presynaptic elements, except for deep tectal afferents. Judging from the number of synapses in the different layers (fig.4 on p.98) no more than 5% of their input is visual and no more dan 5% is telencephalic. At least 90% of their input comes from the torus longitudinalis.

$$i\ (I)=0.05vis+0.9tor+0.05tel \tag{1a}$$

Type III: may be contacted by visual afferents, axons of other type III neurons and axons of type VII neurons (see figs. V.8-V.11). In condition 1 this results in the following formula:

$$i\,(III)=1/3vis+1/3\;o(III)+1/3\;o(VII) \qquad (2a)$$

Type IV: $$i\,(IV)=1/3vis+1/3\;o(IV)+1/3\;o(VIII) \qquad (3a)$$

Type V: consists of two different subpopulations in our schemes, one in layer 4 and one in layer 3, with the following inputs:

$$i\,(V_{l_4})=1/2\;o(V_{l_4})+1/2\;o(IX) \qquad (4a)$$

$$i\,(V_{l_3})=1/3tel+1/3deep+1/3\;o(V_{l_3}) \qquad (5a)$$

Type VII: is contacted by the same axons as type III and IV together. Judging from their dendritic extension, the basal dendrites in layer 4/5 have about 4 times more synapses than the dendrites in layer 5 and 6, in formula:

$$i\,(VII)=1/5\{1/3vis+1/3\;o(III)+1/3\;o(VII)\}+4/5\{1/3vis+1/3\;o(IV)+1/3\;o(VIII)\}=$$

$$i\,(VII)=1/3vis+1/15\;o(III)+4/15\;o(IV)+1/15\;o(VII)+4/15\;o(VIII) \qquad (6a)$$

Type VIII: Judging from the extension of the dendritic trees and the length of the dendritic shaft, the ratio between the importance of the dendrites in layer 4/5, dendritic shaft and soma in layer 4 and dendrites in layer 3/4 is estimated to be 2 : 1 : 1. This results in the following equation:

$$i\,(VIII)=1/2\{1/3vis+1/3\;o(IV)+1/3\;o(VIII)\}+1/4\{1/2\;o(V_{l_4})+1/2\;o(IX)\}+ 1/4\{1/2tel+1/2\;o(I)\}$$

$$i\,(VIII)=1/6vis+1/8tel+1/8\;o(I)+1/6\;o(IV)+1/8\;o(V_{l_4})+1/6\;o(VIII)+1/8\;o(IX) \qquad (7a)$$

Type IX: is contacted by the same types of presynaptic structures as type VIII. However, the ratio between the influence of the dendrites in layer 4/5, those in layer 4 and the dendrites and soma in layer 3/4 is estimated to be 2 : 1 : 2 for type IX, resulting in the following equation:

$$i\,(IX)=2/5\{1/3vis+1/3\;o(IV)+1/3\;o(VIII)\}+1/5\{1/2\;o(V_{l_4})+1/2\;o(IX)\}+ 2/5\{1/2tel+1/2\;o(I)\}=$$

$$i\,(IX)=2/15vis+2/10tel+2/10\;o(I)+2/15\;o(IV)+1/10\;o(V_{l_4})+2/15\;o(VIII)+ 1/10\;o(IX) \qquad (8a)$$

According to assumption 7 (see also general discussion, section 2.3.6) i (cell type) = o (cell type), which means that equation (1) to (8) constitute eight equations with six unknowns. These can be solved in terms of visual, telencephalic, toral and deep tectal input, resulting in the following set of equations(see also fig. V.13):

$$i\,(I)=0.05vis+0.9tor+0.05tel \qquad (1b)$$
$$i\,(III)=0.88vis+0.06tor+0.06tel \qquad (2b)$$
$$i\,(IV)=0.73vis+0.12tor+0.14tel \qquad (3b)$$
$$i\,(V_{l_4})=0.38vis+0.29tor+0.33tel \qquad (4b)$$
$$i\,(V_{l_3})=0.50tel+0.50deep \qquad (5b)$$
$$i\,(VII)=0.76vis+0.11tor+0.13tel \qquad (6b)$$
$$i\,(VIII)=0.47vis+0.25tor+0.29tel \qquad (7b)$$
$$i\,(IX)=0.38vis+0.29tor+0.33tel \qquad (8b)$$

From these equations the input of other tectal neurons can now be estimated.

Type XIV: Judging from the number of synapses (fig. 4, on p.98), the ratio between the influence of the dendrites in layer 5/6, 5 and 4/5 and the dendrites and dendritic shaft in layer 4, 3 and 2 is 2 : 1. The influence of structures in layer 5/6 and 5 compared with those in layer 4/5 is estimated to be proportional to 2 : 1. The influence of structures in layer 4 : 3/4 : 3 : 2 is estimated to be proportional to 2 : 2 : 1 : 1, in formula:

$$i\ (XIV) = (1-\alpha).o(XIV) + \alpha\Big\{2/3\big[2/3\{1/3vis+1/3\ o(III)+1/3\ o(VII)\}+1/3\{1/3vis+ 1/3\ o(IV)+ 1/3\ o(VIII)\}\big]+1/3\big[2/6\{1/2\ o(V_{1_4})+1/2\ o(IX)\}+2/6\{1/2tel+ 1/2\ o(I)\}+1/6\{1/3tel+1/3deep+1/3\ (V_{1_3})\}+1/6deep\big]\Big\} =$$

$$i\ (XIV) = \alpha\ \{0.22vis+0.07tel+0.07deep+0.06\ o(I)+0.15\ o(III)+0.07\ o(IV)+ 0.06\ o(V_{1_4})+0.02\ o(V_{1_3})+0.15\ o(VII)+0.07\ o(VIII)+0.06\ o(IX)\}+ (1-\alpha).o(XIV) \quad (9a)$$

or

$$i\ (XIV) = 0.60vis+0.14tor+0.18tel+0.08deep \quad (9b)$$

Type VI: Judging from the number of synapses (see fig. 4, on p.98), the ratio between the influence of the dendrites in layer 6, the dendritic shaft and soma in layer 5 and 4/5, and the dendrites in layer 4 is 1 : 1 : 2. A detailed discussion on the input of the dendrites in layer 6 as well as in layer 4 is presented in the general discussion , section 2.3.2; in formula:

$$i\ (VI) = 1/4vis+1/4\big[1/2\{1/3vis+1/3\ o(III)+1/3\ o(VII)\}+1/2\{1/3vis+1/3\ o(IV)+ 1/3\ o(VIII)\}\big]+1/2\ o(VIII) =$$

$$i\ (VI) = 0.33vis+0.04\ o(III)+0.04\ o(IV)+0.04\ o(VII)+0.54\ o(VIII) \quad (10a)$$

or

$$i\ (VI) = 0.69vis+0.15tor+0.17tel \quad (10b)$$

Type X: Type X neurons have a similar dendritic morphology as type IX neurons, and consequently receive the same kind of input.

$$i\ (X) = 2/15vis+1/5tel+1/5\ o(I)+2/15\ o(IV)+1/10\ o(V_{1_4})+2/15\ o(VIII)+ 1/10\ o(IX) \quad (11a)$$

or

$$i\ (X) = 0.38vis+0.29tor+0.33tel \quad (11b)$$

Type XII: Judging from the distribution of synapses, the ratio between the influences of structures in layer 5 and 4/5, layer 4, layer 3/4 and 3, and layer 2/3 is 3 : 6 : 5 : 7. The influence of layer 5 compared with layer 4/5 is estimated to be proportional to 2 : 1;in formula:

$$i\ (XII) = 2/21\{1/3vis+1/3\ o(III)+1/3\ o(VII)\}+1/21\{1/3vis+1/3\ o(IV)+1/3\ o(VIII)\} +6/21\{1/2\ o(V_{1_4})+1/2\ o(IX)\}+5/21\{1/3tel+1/3\ o(I)+1/3\ o(V_{1_3})\} +7/21deep =$$

$$i\ (XII) = 0.05vis+0.08tel+0.33deep+0.08\ o(I)+0.03\ o(III)+0.02\ o(IV)+ 0.14\ o(V_{1_4})+0.08\ o(V_{1_3})+0.03\ o(VII)+0.02\ o(VIII)+0.14\ o(IX) \quad (12a)$$

or

$$i\ (XII) = 0.23vis+0.16tor+0.23tel+0.37deep \quad (12b)$$

Type XIII: From the number of synapses and the dendritic extension, the degree of influence of structures in layer 5/6 and 5; layer 4/5; layer 4+3/4; layer 3; and layer 2 is estimated to be proportional to 1 : 1 : 2 : 3 : 5. This yields the following equations:

$$i\ (XIII) = 1/12\{1/3vis+ 1/3\ o(III)+1/3\ o(VII)\}+1/12\{1/3vis+1/3\ o(IV)+ 1/3\ o(VIII)\}+2/12\big[1/2\{1/2\ o(V_{1_4})+1/2\ o(IX)\}+1/2\{1/2tel+1/2\ o(I)\}\big]+ 3/12\{1/3tel+1/3deep+1/3\ o(V_{1_3})\}+5/12deep =$$

$$i\,(XIII)=0.06vis+0.13tel+0.50deep+0.04\ o(I)+0.03\ o(III)+0.03\ o(IV)+0.04\ o(V_{1_4})+0.08\ o(V_{1_3})+0.03\ o(VII)+0.03\ o(VIII)+0.04\ o(IX) \quad (13a)$$

or

$$i\,(XIII)=o.17vis+0.07tor+0.21tel+0.54deep \quad (13b)$$

With respect to the presynaptic elements in each layer, except for type XIV axons, the following equations can be formed in condition 1:

$$i\ (\text{layer } 7)=1.0tor \quad (14a)$$
$$i\ (\text{layer } 6)=1.0vis \quad (15a)$$
$$i\ (\text{layer } 5/6+5)=1/3vis+1/3\ o(III)+1/3\ o(VII) \quad (16a)$$
$$i\ (\text{layer } 4/5)=1/3vis+1/3\ o(IV)+1/3\ o(VIII) \quad (17a)$$
$$i\ (\text{layer } 4)=1/2\ o(IX)+1/2\ o(V_{1_4}) \quad (18a)$$
$$i\ (\text{layer } 3/4)=1/2tel+1/2\ o(I) \quad (19a)$$
$$i\ (\text{layer } 3)=1/3tel+1/3deep+1/3\ o(V_{1_3}) \quad (20a)$$
$$i\ (\text{layer } 2/3+2)=1.0deep \quad (21a)$$

When the equations 1b to 8b are filled in, the following equations can be derived.

$$i\ (\text{layer } 7)=1.0tor \quad (14b)$$
$$i\ (\text{layer } 6)=1.0vis \quad (15b)$$
$$i\ (\text{layer } 5/6+5)=0.88vis+0.06tor+0.06tel \quad (16b)$$
$$i\ (\text{layer } 4/5)=0.73vis+0.12tor+0.14tel \quad (17b)$$
$$i\ (\text{layer } 4)=0.38vis+0.29tor+0.33tel \quad (18b)$$
$$i\ (\text{layer } 3/4)=0.03vis+0.45tor+0.53tel \quad (19b)$$
$$i\ (\text{layer } 3)=0.50tel+0.50deep \quad (20b)$$
$$i\ (\text{layer } 2/3+2)=1.0deep \quad (21b)$$

According to equation 16a and 17a, the SFGS should contain about 33% visual afferents, of which the retinal afferents are quantitatively the most important ones. This is too high a percentage in comparison with the real values found for retinal afferents (about 18%, chapter IVb; Airhart and Kriebel,'80), due to the neglect of type XIV axons.

Condition 2

In this condition the influence of the different types of presynaptic elements is proportional to their frequency of occurrence. The numbers available and the resultant ratios are indicated in table V.1. From these numbers the following equations can be formed for the input in the different tectal layers.

layer 7: $i\ (1_7)=100\%\ tor$ (14c)

layer 6: $i\ (1_6)=$? (100% visual; see general discussion; section 2.3.2) (15c)

layer 5: The ratio between the frequency of occurrence of different types of presynaptic elements is as follows:

o(ret): o(NP): o(AP): o(NRMT): o(NI): o(III): o(VII): o(XIV)=

(2/3x160): 0.4: 0.4: 0.4: 1: 5: 1: (2/9x1200). So,

$$i(1_5)=0.28\ o(ret)+0.001\ o(NP)+0.001(AP)+0.001\ o(NRMT)+0.003\ o(NI)+0.013\ o(III)+0.003\ o(VII)+0.70\ o(XIV)=$$

$$i(1_5)=0.29vis+\ 0.013\ o(III)+0.003\ o(VII)+0.70\ o(XIV) \quad (16c)$$

layer 4/5: o(ret): o(IV): o(VIII): o(XIV)=(1/3x160):1:1:(1/9x1200). So,

$$i\ (1_{4/5})=0.28\ o(ret)+0.005\ o(IV)+0.005\ o(VIII)+0.71\ o(XIV) \quad (17c)$$

Table V.1
Frequency of occurrence and laminar distribution of presynaptic elements in the goldfish tectum

type of presynaptic element	*frequency of occurrence*	*ratio number*	*distribution over the tectal layers*[1]
axons of type I neurons	12.500[2]	10	layer 3/4
axons of type III neurons	6.250[2]	5	layer 5
axons of type IV-XIII neurons, each	1.250[2]	1	see fig. 7
axons of type XIV neurons	1500.000[2]	1200	layer 5:4/5:4:3/4:3=2:1:3:1:2
afferents from the retina	200.000[2]	160	layer 5:4/5=2:1
afferents from the TL	100.000[3]	80	layer 7
afferents from the telenc.	?	(10) [4]	layer 3/4:layer 3=1:1
deep tectal afferents	?	(10) [4]	layer 3:2/3:2=1:1:2
afferents from NP	500[3]	0.4	layer 5
afferents from AP	500[3]	0.4	layer 5
afferents from NRMT	?	(0.4)[5]	layer 5
afferents from NI	1250[6]	1	layer 5

[1] roughly estimated on the basis of the width of these layers and/or the morphology of the axons
[2] see chapter III of the present thesis
[3] derived from cell counts in Nissl-stained material; see also General discussion section 1.1 and 1.4
[4] ratio number has arbitrarily been set equal to type I axons
[5] ratio number has arbitrarily been set equal to pretectal nuclei
[6] derived from cell counts in Nissl-stained material

layer 4: $o(V_{1_4}) : o(IX) : o(XIV) = 0.5:1:(3/9 \times 1200)$. So,

$$i(1_4) = 0.001\ o(V_{1_4}) + 0.002\ o(IX) + 0.996\ o(XIV) \quad (18c)$$

layer 3/4: $o(tel) : o(I) : o(XIV) = (1/2 \times 10):10:(1/9 \times 1200)$. So,

$$i(1_{3/4}) = 0.04 tel + 0.07\ o(I) + 0.90\ o(XIV) \quad (19c)$$

layer 3: $o(tel) : o(deep) : o(V_{1_3}) : o(XIV) = (1/2 \times 10):(1/3 \times 10):0.5:(2/9 \times 1200)$. So,

$$i(1_3) = 0.03 tel + 0.02 deep + 0.003\ o(V_{1_3}) + 0.95\ o(XIV) \quad (20c)$$

layer 2/3+2: $i(1_{2+2/3}) = 100\%$ "deep" (21c)

From the numbers presented in table V.1, the input-equations of tectal neurons can be derived in the same way as in condition 1. This would yield equations 1c to 13c. These equations, however, will not be written out here, since they are not used in the general discussion section. The reason for this is that the equations 14c to 21c show already clearly that this condition is not realistic. On the one hand, structures with a low frequency of occurrence (tectal cell types IV, V, VI, VIII, IX, afferents from NP, AP, NI and NRMT), have almost no influence, which would seem to make them redundant. On the other hand, structures with a high frequency of occurrence (retinal fibers and type XIV neurons) have too large an influence in condition 2. Retinal fibers would constitute about 28% of the presynaptic elements in layer 5 and 4/5, which is too high as compared with values measured (about 18%, Airhart and Kriebel, '80; chapter IVb). Type XIV axons would constitute about 90% of all tectal presynaptic elements. However, a value of about 50% seems more realistic, since this is the frequency of occurrence of S5 terminals in the tectum of

Holocentrus (Ito et al., '80), which are presumably for the major part constituted by type XIV axon terminals (see discussion section of chapter IVa). So, in tectal circuitry a relatively large frequency of occurrence of a certain type of presynaptic structure seems to a certain extent to be compensated by a relatively low number of synapses made by each individual structure, and vice versa.

Condition 3

This condition lies in between the extreme conditions 1 and 2. For type XIV axons it is assumed that they constitute 50% of contacts in layer 5 and 5/6; 25% of all contacts in layer 4/5; 75% of all contacts in layer 4; 33% of all contacts in layer 3/4 and 50% of all contacts in layer 3. This distribution is based on the following considerations. The percentages enumerated result in an average percentage of about 50%, as is in agreement with the percentage of S5 terminals in the tectum of *Holocentrus* (Ito et al., '80), which are probably constituted for the major part by type XIV axon terminals (see discussion section of chapter IVa). The axons of type XIV neurons have no specific preference to terminate in the narrow layers 4/5 and 3/4, and are assumed to have in these layers the same amount of influence as the other types of presynaptic elements (in layer 4/5: visual afferents, axons of type IV neurons and axons of type VII neurons; in layer 3/4: telencephalic afferents and axons of type I neurons). Their influence in the wider layers 5, 4 and 3 is assumed to be larger. The ratio between 50% (layer 5); 75% (layer 4) and 50% (layer 3) roughly reflects the preference of type XIV to terminate in layer 4(see chapter III and also the results calculated for condition 2); and is also in accordance with the distribution of S5 terminals in *Holocentrus* (Ito et al., '80). It should be mentioned furthermore that the resultant percentage of contacts of visual afferents in layer 5 and 4/5 is about 16% and 25%, resulting in an average value of about 19% for the whole SFGS. This is in line with the values found for retinal fibers (about 18%, see Airhart and Kriebel, '80; chapter IVb). So, with respect to the number and laminar distribution of type XIV axon terminals, condition 3 represents the most probable estimation that can be made at present. However, with respect to the preference of type XIV axons to make contacts with type XIV dendrites, the reality is probably situated somewhere in between condition 1 and 3. Condition 1 assumes 100% preferences and condition 3 0%, which both seem too extreme (see general discussion section 2.3.6). The following equations hold in condition 3 (for further details the reader is referred to condition 1).

Type I: i (I)=0.05vis+0.9tor+0.04tel+0.01deep (is set equal to condition 1, except for one percent of deep input via type XIV axons) (1d)

Type III: i (III)=1/2 o(XIV)+1/2{1/3vis+1/3 o(III)+1/3 o(VII)}=

i (III)=0.17vis+0.17 o(III)+0.17 o(VII)+0.50(XIV) (2d)

Type IV: i (IV)=1/4vis+1/4 o(IV)+1/4 o(VIII)+1/4 o(XIV) (3d)

Type V_{1_4}: i (V_{1_4})=3/4 o(XIV)+1/4{1/2 o(V_{1_4})+1/2 o(IX)}=

i (V_{1_4})=0.13 o(V_{1_4})+0.13 o(IX)+0.75 o(XIV) (4d)

Type V_{1_3}: i (V_{1_3})=1/2 o(XIV)+1/2{1/3tel+1/3deep+1/3 o(V_{1_3})}=

i (V_{1_3})=0.17tel+0.17deep.0.17 o(V_{1_3})+0.50 o(XIV) (5d)

Type VII: i (VII)= 1/5[1/2 o(XIV)+1/2{1/3tor+1/3 o(III)+1/3 o(VII)}] +
4/5[1/4vis+1/4 o(IV)+1/4 o(VIII)+1/4 o(XIV)] =

i (VII)=0.23vis+0.03 o(III)+0.20 o(IV)+0.03 o(VII)+ 0.20 o(VIII)+0.30 o(XIV) (6d)

Type VIII:

$$i\ (VIII)=1/2\left[1/4vis+1/4\ o(IV)+1/4\ o(VII)+1/4\ o(XIV)\right]+1/4\left[3/4\ o(XIV)+1/4\{1/2\ o(V_{1_4})+1/2\ o(IX)\}\right]+1/4\left[1/3\ o(XIV)+1/3\ o(I)+1/3tel\right]=$$

$$i\ (VIII)=0.13vis+0.08tel+0.08\ o(I)+0.13\ o(IV)+0.03\ o(V_{1_4})+0.13\ o(VIII)+0.03\ o(IX)+0.40\ o(XIV) \quad (7d)$$

Type IX:

$$i\ (IX)=2/5\left[1/4vis+1/4\ o(IV)+1/4\ o(VIII)+1/4\ o(XIV)\right]+1/5\left[3/4\ o(XIV)+1/4\{1/2\ o(V_{1_4})+1/2\ o(IX)\}\right]+2/5\left[1/3\ o(XIV)+1/3tel+1/3\ o(I)\right]=$$

$$i\ (IX)=0.10vis+0.13tel+0.13\ o(I)+0.10\ o(IV)+0.03\ o(V_1)+0.10\ o(VIII)+0.03\ o(IX)+0.38\ o(XIV) \quad (8d)$$

Type XIV:

$$i\ (XIV)=2/3\Big\{2/3\left[1/2\ o(XIV)+1/2\{1/3vis+1/3\ o(III)+1/3\ o(VII)\}\right]+1/3\left[1/4vis+1/4\ o(IV)+1/4\ o(VIII)+1/4\ o(XIV)\right]\Big\}+1/3\Big\{2/6\left[3/4\ o(XIV)+1/4\{1/2\ o(V_{1_4})+1/2\ o(IX)\}\right]+2/6\left[1/3\ o(XIV)+1/3tel+1/3\ o(I)\right]+1/6\left[1/2\ o(XIV)+1/2\{1/3tel+1/3deep+1/3\ o(V_{1_3})\}\right]+1/6deep\Big\}=$$

$$i\ (XIV)=0.13vis+0.05tel+0.07deep.0.04\ o(I)+0.07\ o(III)+0.06\ o(IV)+0.01\ o(V_{1_4})+0.01\ o(V_{1_4})+0.07\ o(VII)+0.06\ o(VIII)+0.01\ o(IX)+0.43\ o(XIV) \quad (9d)$$

Assuming that i(cell type)= o(cell type) (see under condition 1), the equations 1d to 9d constitute nine equations with eight unknowns, which can be solved in terms of visual, toral, telencephalic and deep tectal input; for type I it was assumed that they could receive some deep information via the axons of type XIV. The following set of equations results:

$$i\ (I)=0.05vis+0.90tor+0.04tel+0.01deep \quad (1e)$$
$$i\ (III)=0.67vis+0.09tor+0.12tel+0.12deep \quad (2e)$$
$$i\ (IV)=0.70vis+0.09tor+0.12tel+0.09deep \quad (3e)$$
$$i\ (V_{1_4})=0.54vis+0.13tor+0.17tel+0.16deep \quad (4e)$$
$$i\ (V_{1_3})=0.33vis+0.07tor+0.30tel+0.30deep \quad (5e)$$
$$i\ (VII)=0.69vis+0.09tor+0.12tel+0.10deep \quad (6e)$$
$$i\ (VIII)=0.54vis+0.16tor+0.20tel+0.10deep \quad (7e)$$
$$i\ (IX)=0.47vis+0.20tor+0.25tel+0.09deep \quad (8e)$$
$$i\ (XIV)=0.56vis+0.12tor+0.16tel+0.17deep \quad (9e)$$

From these equations, the input of the efferent tectal neurons can also be estimated.

Type VI:

$$i\ (VI)=1/4vis+1/4\left[1/2\{1/2\ o(XIV)+1/6vis+1/6\ o(III)+1/6\ o(VII)\}+1/2\{1/4vis+1/4\ o(IV)+1/4\ o(VIII)+1/4\ o(XIV)\}\right]+1/2\left[3/4\ o(XIV)+1/4\ o(VIII)\right]=$$

$$i\ (VI)=0.30vis+0.02\ o(III)+0.03\ o(IV)+0.02\ o(VII)+0.16\ o(VIII)+0.47\ o(XIV) \quad (10d)$$

or

$$i\ (VI)=0.70vis+0.09tor+0.12tel+0.10deep \quad (10e)$$

Type X: the equations for type X are equal to those of type IX,

$$i\ (X)=0.10vis+0.13tel+0.13\ o(I)+0.10\ o(IV)+0.03\ o(V_{1_4})+0.10\ o(VIII)+0.03\ o(IX)+0.38\ o(XIV) \quad (11d)$$

or

$$i\ (X)=0.47vis+0.20tor+0.25tel+0.09deep \quad (11e)$$

Type XII:

$$i\,(XII)=2/21\left[1/2\ o(XIV)+1/2\{1/3vis+1/3\ o(III)+1/3\ o(VII)\}\right]+1/21\left[1/4vis+1/4\ o(IV)+1/4\ o(VIII)+1/4\ o(XIV)\right]+6/21\left[3/4\ o(XIV)+1/8\ o(V_{1_4})+1/8\ o(IX)\right]+5/21\left[1/2\{1/3tel+1/3\ o(I)+1/3\ o(XIV)\}+1/2\ \{1/2\ o(XIV)+1/6tel+1/6deep+1/6\ o(V_{1_3})\}\right]+7/21\ deep=$$

$$i\,(XII)=0.03vis+0.06tel+0.35deep+0.04\ o(I)+0.02\ o(III)+0.01\ o(IV)+0.04\ o(V_{1_4})+0.02\ o(V_{1_3})+0.02\ o(VII)+0.01\ o(VIII)+0.04\ o(IX)+0.37\ o(XIV) \qquad (12d)$$

or

$$i\,(XII)=0.32vis+0.10tor+0.15tel+0.44deep \qquad (12e)$$

Type XIII:

$$i\,(XIII)=1/12\left[1/2\ o(XIV)+1/2\{1/3vis+1/3\ o(III)+1/3\ o(VII)\}\right]+1/12\left[1/4vis+1/4\ o(IV)+1/4\ o(VIII)+1/4\ o(XIV)\right]+2/12\left[1/2\{3/4\ o(XIV)+1/8\ o(V_{1_4})+1/8\ o(IX)\}+1/2\{1/3tel+1/3\ o(I)+1/3\ o(XIV)\}\right]+3/12\left[1/2\ o(XIV)+1/2\{1/3tel+1/3deep+1/3\ o(V_{1_3})\}\right]+5/12deep=$$

$$i\,(XIII)=0.04vis+0.07tel+0.46deep+0.03\ o(I)+0.01\ o(III)+0.02\ o(IV)+0.01\ o(V_{1_4})+0.04\ o(V_{1_3})+0.01\ o(VII)+0.02\ o(VIII)+0.01\ o(IX)+0.28\ o(XIV) \qquad (13d)$$

or

$$i\,(XIII)=0.26vis+0.07tor+0.14tel+0.53deep \qquad (13e)$$

With respect to the presynaptic elements in each layer, the following equations can be formed in condition 3:

$$i\,(layer\ 7)=1.0tor \qquad (14d)$$
$$i\,(layer\ 6)=1.0vis \qquad (15d)$$
$$i\,(layer\ 5/6+5)=1/6vis+1/6\ o(III)+1/6\ o(VII)+1/2\ o(XIV) \qquad (16d)$$
$$i\,(layer\ 4/5)=1/4vis+1/4\ o(IV)+1/4\ o(VII)+1/4\ o(XIV) \qquad (17d)$$
$$i\,(layer\ 4)=1/8\ o(V_{1_4})+1/8\ o(IX)+3/4\ o(XIV) \qquad (18d)$$
$$i\,(layer\ 3/4)=1/3tel+1/3\ o(I)+1/3\ o(XIV) \qquad (19d)$$
$$i\,(layer\ 3)=1/6tel+1/6deep+1/6\ o(V_{1_3})+1/2\ o(XIV) \qquad (20d)$$
$$i\,(layer\ 2/3+2)=1.0deep \qquad (21d)$$

When equations 1e to 9e are filled in, the following equations can be derived:

$$i\,(layer\ 7)=1.0tor \qquad (14e)$$
$$i\,(layer\ 6)=1.0vis \qquad (15e)$$
$$i\,(layer\ 5/6+5)=0.67vis+0.09tor+0.12tel+0.12deep \qquad (16e)$$
$$i\,(layer\ 4/5)=0.70vis+0.09tor+0.12tel+0.09deep \qquad (17e)$$
$$i\,(layer\ 4)=0.54vis+0.13tor+0.17tel+0.16deep \qquad (18e)$$
$$i\,(layer\ 3/4)=0.20vis+0.34tor+0.40tel+0.06deep \qquad (19e)$$
$$i\,(layer\ 3)=0.33vis+0.07tor+0.30tel+0.30deep \qquad (20e)$$
$$i\,(layer\ 2/3+2)=1.0deep \qquad (21e)$$

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VI SUMMARY

The purpose of the present thesis is to increase insight in the functional anatomy of the tectum mesencephali of the goldfish on the basis of detailed histological observations at the light- and electron microscopical level concerning the somatic, dendritic, axonal and synaptic properties of tectal neurons. For this purpose a qualitative and quantitative Golgi- and combined Golgi-electron microscopic investigation has been performed.

By means of the Golgi technique fifteen cell types could be distinguished, primarily based upon their characteristic dendritic lamination patterns. Several aspects of these cell types have been quantified, including the lamination patterns and extensions of their dendritic trees, the locations and dimensions of their cell bodies and their frequency of occurrence. Four cell types are provided with a myelinated axon that leaves the tectum, eight cell types have an unmyelinated axon terminating at characteristic levels within the tectum, and three cell types have obscure axonal properties. In comparison with the number of optic nerve fibers terminating within the tectum(about 200.000), the number of efferent tectal neurons is relatively small(2000-8000), whereas the total number of tectal interneurons is relatively high (1-2 million).

For the combined Golgi-electron microscopical investigation three types of interneurons and three types of efferent neurons were selected. The main results of this investigation are:1) Dendrites and cell bodies appear to be exclusively postsynaptic structures whereas axon terminals are exclusively presynaptic structures. 2) The cell types investigated each have a characteristic distribution of synaptic density along the surface of their dendrites and cell bodies, resulting in characteristic mean numbers of synapses per postsynaptic component. 3) Optic nerve fibers contribute to about 10-20% of the synaptic contacts on the postsynaptic structures studied in layer 5.

In the general discussion a functional anatomical framework concerning the tectum mesencephali is constructed on the basis of: 1) the lamination pattern of tectal afferents as described in the literature; 2) the lamination pattern of postsynaptic and presynaptic structures of tectal cell types as determined by the present Golgi-study; 3) the number of synapses on the cell types studied by means of the Golgi-electron microscopic technique and 4)a number of assumptions concerning the contribution of the different types of presynaptic structures present in each tectal layer in the formation of synapses. On the basis of this framework estimations are presented for the flow of information from the different types of tectal input through the neuronal tectal circuit and for the importance of the different types of tectal neurons in the processing of signals from the different types of tectal afferent systems.

It may be concluded that the topographical organization of the tectum is aimed at the localization of objects in the environment of the animal and the generation of goal-directed movements, whereas the laminar organization of the tectum is relevant for the selection of significant objects on the basis of multimodal information.

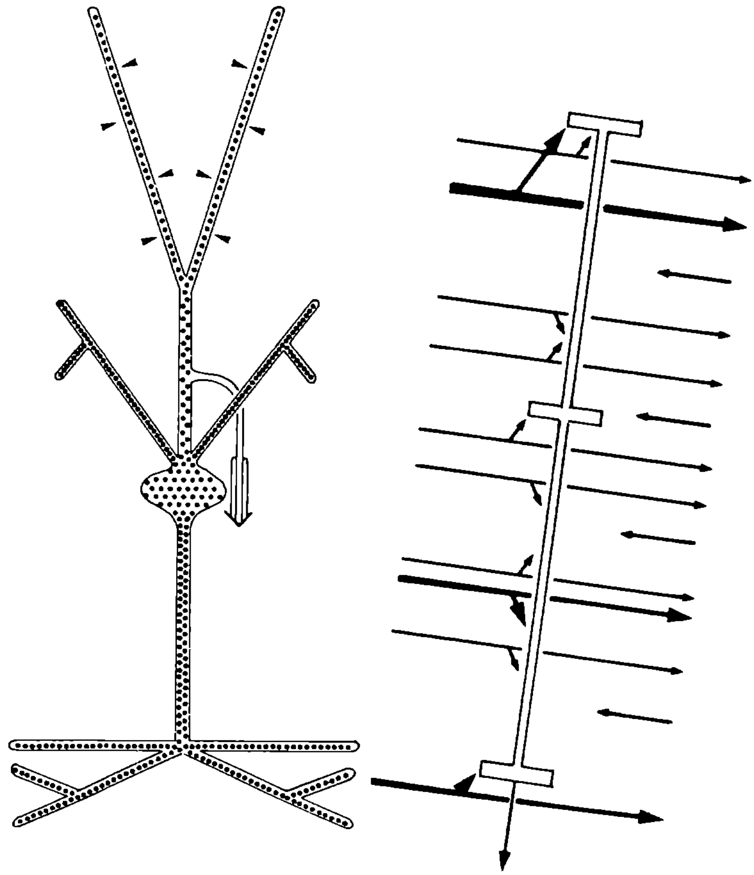
CURRICULUM VITAE

Johannes Meek werd 6 juli 1949 geboren te Utrecht. Na het behalen van het Gymnasium-B diploma in 1967 studeerde hij biologie aan de Rijksuniversiteit te Utrecht. In september 1974 behaalde bij het doctoraaldiploma. Van oktober 1974 tot november 1978 werkte hij als wetenschappelijk medewerker in dienst van Z.W.O. op het Laboratorium voor Medische Fysica der Universiteit van Amsterdam. Van november 1978 tot mei 1979 was hij wetenschappelijk ambtenaar in dienst van het Interuniversitair Oogheelkundig Instituut te Amsterdam. Sinds augustus 1979 is hij in dienst van het Laboratorium voor Anatomie en Embryologie van de Katholieke Universiteit te Nijmegen.

HET TECTUM MESENCEPHALI VAN DE GOUDVIS

(CARASSIUS AURATUS)

Een licht- en elektronenmikroskopisch onderzoek



SAMENVATTING van het proefschrift "THE TECTUM MESENCEPHALI OF THE GOLDFISH (CARASSIUS AURATUS). A light- and electron microscopic investigation". Katholieke Universiteit Nijmegen, 1981

J. Meek

SAMENVATTING

Inleidende opmerkingen

Het onderzoek dat in dit proefschrift wordt beschreven betreft een onderzoek naar de struktuur van het dak van de middenhersenen, het *tectum mesencephali*, van de goudvis. Als we de schedel van een goudvis openen, kunnen we de hersenen van bovenaf zien liggen (fig. A). Verschillende delen kunnen worden onderscheiden, o.a. de voorhersenen, middenhersenen, kleine hersenen en achterhersenen (fig. A). Het gedeelte van de middenhersenen dat van bovenaf zichtbaar is is het tectum of dak, en daar is het in dit proefschrift om begonnen. Wanneer we de middenhersenen dwars doorsnijden en het snijvlak door een mikroskoop bekijken, dan zien we dat het tectum inderdaad als een dak boven de rest van de middenhersenen is gelegen (fig. B). Bij mikroskopisch onderzoek valt direkt op dat het tectum is opgebouwd uit lagen. Er kunnen zeven van deze lagen worden onderscheiden, die van binnen naar buiten van 1 $^{t}/_{m}$ 7 zijn genummerd (fig. B).

Het is reeds lang bekend dat de oogzenuw voornamelijk in het tectum eindigt, en dat het tectum dientengevolge betrokken is bij de verwerking van lichtprikkels. De verbinding tussen de ogen en het tectum is *topografisch* geordend, hetgeen betekent dat elk plekje van de retina, het lichtgevoelige netvlies achter in de oogbol, verbonden is met een specifiek plekje van het tectum, met als gevolg dat via de oogzenuw een kaart van de zichtbare buitenwereld op het tectum wordt geprojecteerd. Het in dit proefschrift beschreven onderzoek is vooral opgezet om meer inzicht te verkrijgen in de wijze waarop oogzenuwvezels hun invloed in het tectum uitoefenen.

Bij onderzoek naar de struktuur van een hersencentrum staan de volgende begrippen centraal. Hersenen zijn opgebouwd uit *zenuwcellen* of *neuronen*. Deze zien er uit als bolletjes met een aantal langere of kortere takken. Het bolletje wordt *cellichaam* of *soma* genoemd en er worden twee soorten takken onderscheiden: *dendrieten* of *axonen*. In het algemeen heeft een zenuwcel meerdere dendrieten, doch slechts één axon of zenuwvezel. Het oppervlak van het cellichaam en van de dendrieten is gericht op het ontvangen van signalen, terwijl het axon signalen wegzendt. Daartoe maakt een axon kontakten met andere cellen, zowel met hun dendrieten als met hun cellichaam. Overal waar zo'n kontakt wordt gevormd bevindt zich een struktuurtje dat *synaps* wordt genoemd, en dat het overbrengen van signaaltjes van de ene naar de andere zenuwcel mogelijk maakt. Hersenweefsel bestaat dus voornamelijk uit neuronen met hun uitlopers, en deze uitlopers vormen een zeer ingewikkeld netwerk met grote aantallen onderlinge synaptische kontakten. Wanneer we dit netwerk willen analyseren, hetgeen in dit proefschrift het geval is voor het tectum mesencephali, gaat het dus vooral om een nadere bestudering van de cellichamen, dendrieten, axonen en synapsen van de aanwezige neuronen.

De zojuist genoemde strukturen kunnen slechts met behulp van bepaalde technische hulpmiddelen zichtbaar worden gemaakt. Om cellichamen, dendrieten en axonen te kunnen waarnemen is een *lichtmikroskoop* nodig, een instrument dat

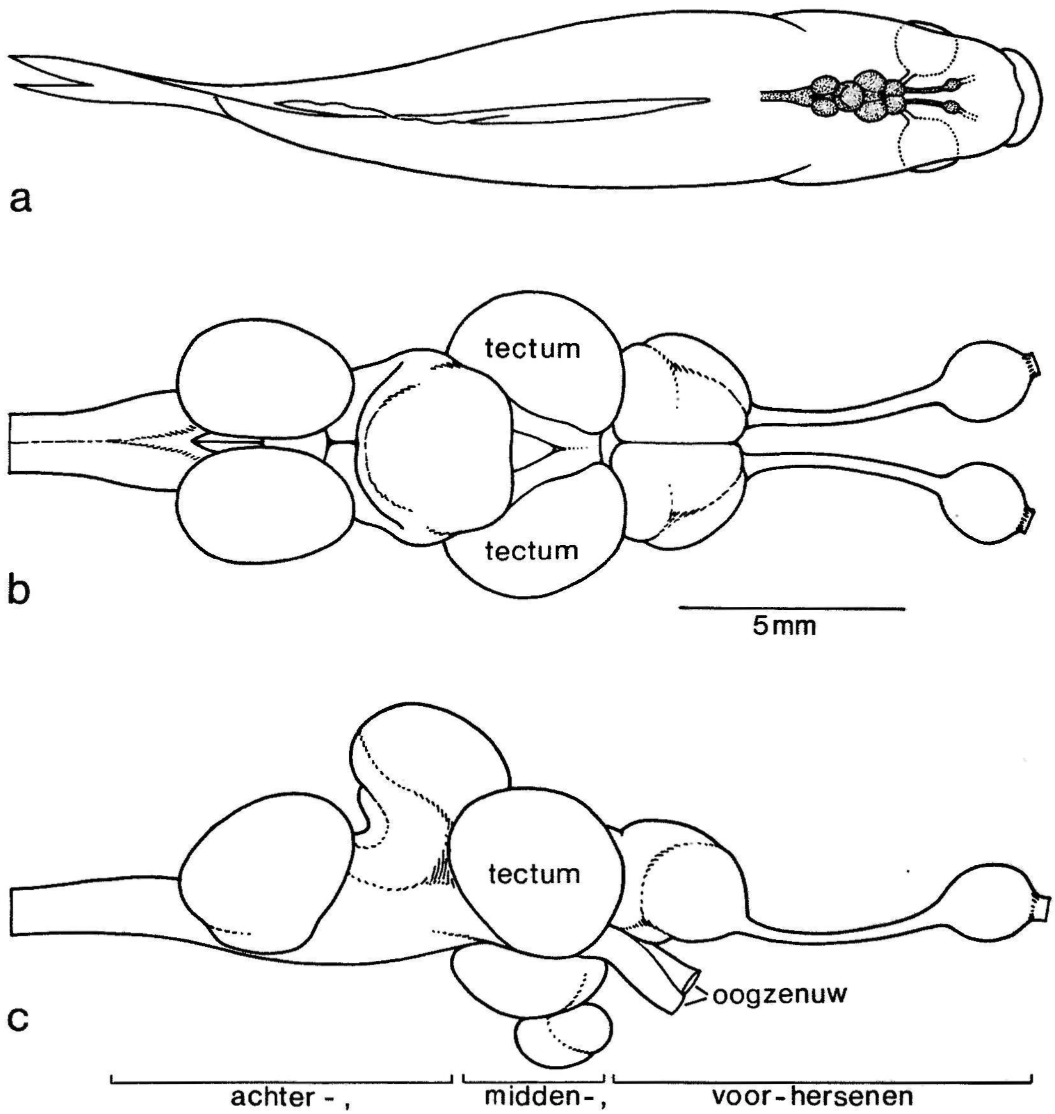


Fig. A. (=Fig. I.1. van het proefschrift) De hersenen van de goudvis. a: De positie van de hersenen in de vis; b: Bovenaanzicht; c: Zijaanzicht.

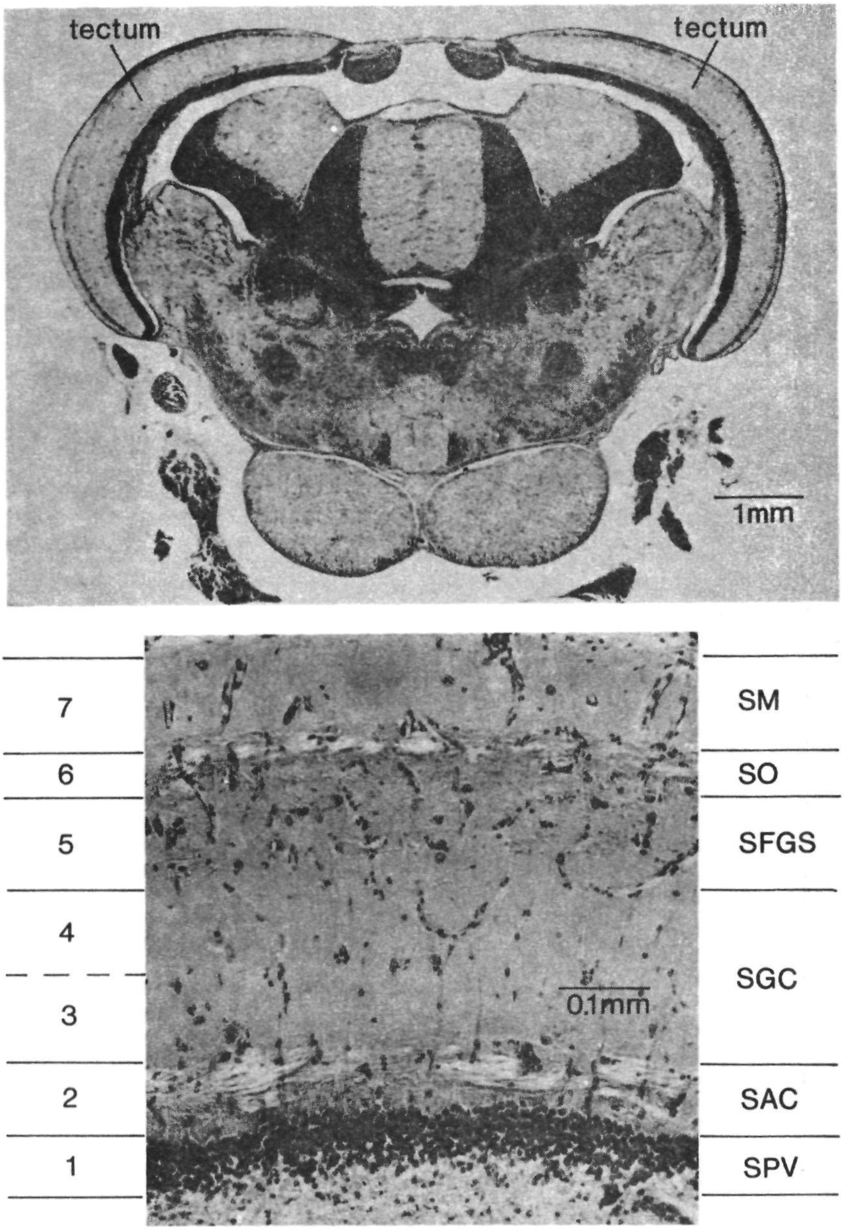


Fig. B. (=Fig. I.2. van het proefschrift) Dwarse doorsneden door het tectum van de goudvis. a: Een opname met een lage vergroting om de ligging van het tectum boven de rest van de hersenen te tonen; b: Een opname met een hogere vergroting om de tectale lagen aan te duiden. SAC: stratum album centrale; SFGS: stratum fibrosum et griseum superficiale; SGC: stratum griseum centrale; SM: stratum marginale; SO: stratum opticum; SPV: stratum periventriculare.

tot ongeveer 1000 x kan vergroten, terwijl voor de bestudering van de kleine synapsen zelfs een *elektronenmikroskoop* nodig is, een instrument dat tot ongeveer 100.000 x kan vergroten. Verder moet hersenweefsel eerst "gefixeerd", dat wil zeggen vastgelegd worden met behulp van chemicaliën, omdat dood weefsel anders zeer snel zijn struktuur verliest en uitelkaar valt. Daarna moeten zeer dunne plakjes weefsel (coupes) worden gesneden om de inwendige struktuur van de hersenen zichtbaar te maken. Voor lichtmikroskopie varieert de vereiste dikte van 0.01 tot 0.1 mm, terwijl voor elektronenmikroskopie de coupes slechts 0.0001 mm dik mogen zijn. Coupes worden gemaakt met een mikrotoom of ultramikrotoom, en het weefsel moet hiervoor worden ingebed in paraffine, celloïdine of plastic, omdat het anders niet hard genoeg is om dunne coupes te snijden. Tenslotte moet een weefselkleuring worden toegepast, omdat ongekleurde coupes bijna geheel transparant zijn. Hiervoor is een groot aantal kleurstoffen beschikbaar. In dit proefschrift wordt voornamelijk de *Golgi-impregnatie* gebruikt, een methode waarmee weliswaar slechts een klein aantal neuronen zichtbaar wordt gemaakt, maar dan wel kompleet met hun cellichaam, dendrieten en axon. Voor elektronenmikroskopie is bovendien een overzichtskleuring gebruikt om synapsen zichtbaar te maken.

Enkele andere begrippen die in de nu volgende samenvatting voorkomen kunnen als volgt worden omschreven:

afferenten: axonen (zenuwvezels) die signalen naar een bepaald hersencentrum toezenden

collateralen: zijtakken van een axon

efferenten: axonen (zenuwvezels) die signalen uit een bepaald hersencentrum wegzenden

elektrofysiologisch onderzoek: onderzoek naar de elektrische signalen van zenuwcellen

impregneren: het vullen van een zenuwcel met een bepaald neerslag

input: het geheel van binnenkomende signalen

kwalitatief morfologisch onderzoek: onderzoek dat gericht is op het *beschrijven* van een bepaalde struktuur

kwantitatief morfologisch onderzoek: onderzoek dat gericht is op het *meten* van een aantal grootheden in een bepaalde struktuur

laminatiepatroon (laminaire organisatie): de wijze waarop een hersencentrum is opgebouwd uit lagen

motoriek: het geheel van bewegingen dat een dier kan uitvoeren

multimodaal: betrekking hebbend op meerdere modaliteiten, in dit geval zintuigsystemen

postsynaptische struktuur: struktuur die zich achter (post-) een synaps bevindt, en dus signalen ontvangt (i.h.a. dendrieten en cellichamen)

presynaptische struktuur: struktuur die zich vóór (pre-) een synaps bevindt, en dus signalen wegzendt (i.h.a. axoneindigingen)

receptief oppervlak: het oppervlak van postsynaptische structuren dat betrokken is bij de vorming van synapsen

responsie: reaktie van een zenuwcel op een bepaalde prikkeling
visueel: betrekking hebbend op licht

Samenvatting

Het in dit proefschrift beschreven onderzoek heeft als hoofddoel het verkrijgen van inzicht in de funktionele anatomie, d.w.z. de samenhang tussen struktuur en funktie, van het tectum mesencephali van de goudvis met behulp van gedetailleerd histologisch onderzoek op licht- en elektronenmikroskopisch niveau. Dit doel, alsmede het verloop van het onderzoek,wordt in de algemene inleiding nader omschreven, waarbij tevens een overzicht wordt gegeven van de literatuur over de struktuur en de funktie van het tectum mesencephali van beenvissen (teleostei) die voor en tijdens het eigen onderzoek werd gepubliceerd. Daarna wordt in hoofdstuk II een enigszins gewijzigde Golgi-methode beschreven die geschikt is gebleken om tectale neuronen volledig te impregneren.

De lichtmikroskopische resultaten worden beschreven in hoofdstuk III. Met behulp van de in hoofdstuk II beschreven Golgi-Cox modifikatie, alsmede met behulp van andere Golgi-methoden konden 15 verschillende celtypen in het tectum van de goudvis worden onderscheiden. Deze celtypen, genummerd van I t/m XV, zijn schematisch weergegeven in figuur C. De celtypen verschillen onderling in vorm, ligging en aantal uitlopers van het cellichaam, en in dendritische en axonale eigenschappen. De dendrietbomen van elk celtype blijken in specifieke tectale lagen te zijn gelegen en daar een karakteristieke uitbreiding te hebben (zie fig. C). Vier celtypen hebben een axon dat het tectum verlaat om in een ander hersencentrum te eindigen (efferente neuronen, type VI, X, XII en XIII), terwijl acht celtypen een axon hebben dat in één of meerdere specifieke lagen binnen het tectum zelf eindigt (interneuronen, type I, III, IV, V, VII, VIII, IX en XIV). Het axon van de drie resterende celtypen is niet gevonden. Het geschatte aantal cellen per type is als volgt: type I: 5000-20.000; type III: 2500-10.000; type IV t/m XIII, elk 500-2000; type XIV: 1-2 miljoen. Vergeleken met het aantal oogzenuwvezels dat in het tectum eindigt, ongeveer 200.000, is het aantal cellen van de typen I t/m XIII relatief klein en hetzelfde geldt voor het totale aantal tectale efferenten, geschat op 2000-8000. Het aantal neuronen behorende tot type XIV is daarentegen relatief groot.

Het elektronenmikroskopisch onderzoek, beschreven in hoofdstuk IV, heeft zich geconcentreerd op een analyse van de synapsen van tectale neuronen met behulp van een gekombineerde Golgi-elektronen mikroskopische techniek. Gezien de grote bewerkelijkheid van deze analyse is het onderzoek beperkt tot zes celtypen, te weten de interneuronen type I, III en XIV (dit zijn de meest talrijke tectale neuronen) en de efferente neuronen type VI, XII en XIII. Uit een kwalitatief onderzoek (hoofdstuk IVa) blijkt dat de onderzochte cellichamen en dendrieten alle uitsluitend postsynaptisch en dus signaal ontvangend zijn, terwijl alle onderzochte axoneindigingen presynaptisch en dus signaal ver-

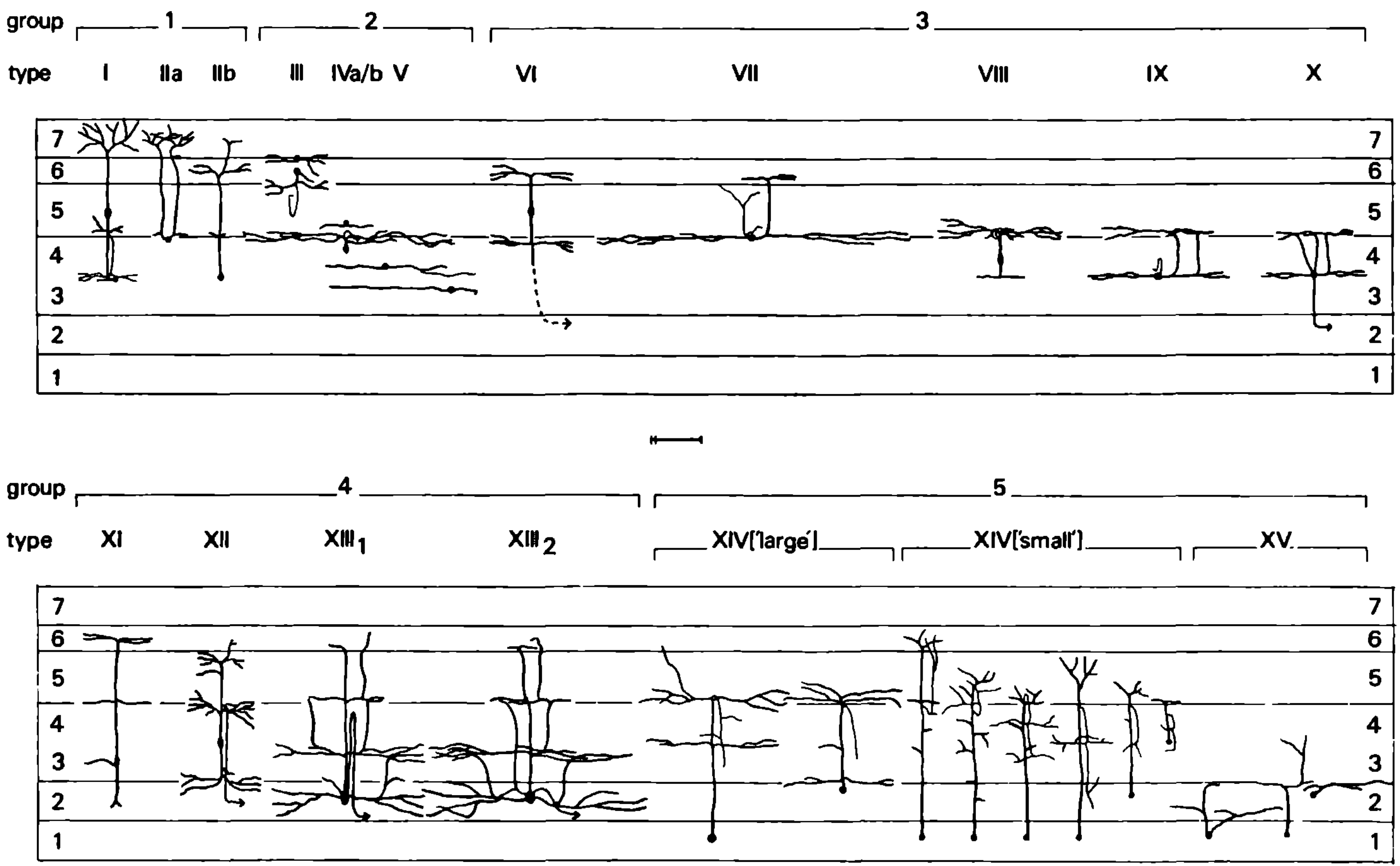


Fig. C. (=Fig. 19 op pag. 51 van het proefschrift). Schematische weergave van de 15 tectale celtypen die in het proefschrift worden beschreven. De afmetingen van de cellichamen en de horizontale uitbreiding van de dendrieten zijn op schaal weergegeven (vergroting: 70x).

schaffend zijn. Verscheidene op ultrastruktureel niveau te onderscheiden tectale afferenten,waaronder oogzenuwvezels, blijken op de onderzochte celtypen te synapteren. De resultaten zijn samengevat in figuur D.Een zeer merkwaardige konfiguratie die is waargenomen betreft het kontakt tussen het boogvormige axon van type $XIII_1$ neuronen en eveneens boogvormig verlopende dendrieten.

Naast dit kwalitatieve onderzoek zijn de synapsen van de geselekteerde tectale neuronen aan een kwantitatieve analyse onderworpen met de nadruk op hun dichtheid, aantal en grootte (hoofdstuk IVb).Tectale celtypen blijken een karakteristieke dichtheidsverdeling van synapsen over hun receptieve oppervlak te bezitten, resulterend in eveneens karakteristieke gemiddelde aantallen per hoofddendriet, dendrietboom en cellichaam. Deze gevonden waarden zijn weergegeven in figuur E. De grootte van de synapsen is niet karakteristiek voor de diverse postsynaptische onderdelen der celtypen, doch staat hoofdzakelijk in verband met het tectale laminatiepatroon en met presynaptische strukturen. Oogzenuwvezels vormen 10 tot 20% van de synaptische kontakten die in laag 5 op

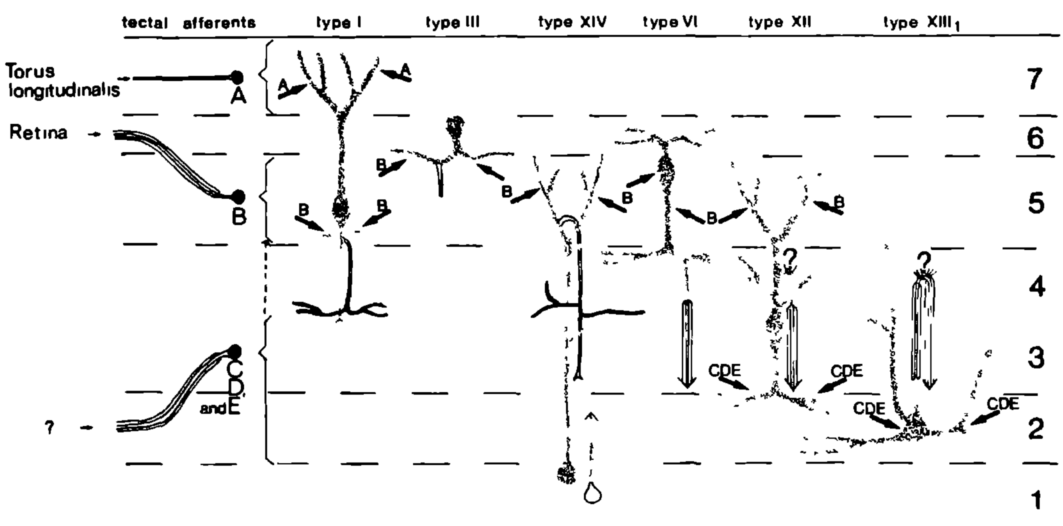


Fig. D. (=Fig. 52 op pag. 87 van het proefschrift). Samenvatting van het kwalitatieve Golgi-elektronen mikroskopische onderzoek. Zwarte strukturen zijn presynaptisch, grijze strukturen zijn postsynaptisch en witte strukturen zijn niet betrokken bij de vorming van synapsen. A, B, C, D en E zijn in het proefschrift beschreven tectale afferenten.

het receptieve oppervlak van de onderzochte neuronen voorkomen (zie fig. E).

In de algemene diskussie (hoofdstuk V) wordt besproken op welke wijze de gevonden resultaten bijdragen tot ons inzicht in het funktioneren van het tectum mesencephali van beenvissen. Uit een overzicht van de literatuur betreffende tectale afferentie blijkt dat behalve de retina tenminste tien hersencentra op het tectum projekteren. Op grond van deze afferentie kunnen vier tectale hoofdzones worden onderscheiden: 1) laag 7 met afferentie uit de torus longitudinalis, een hersencentrum dat alleen bij beenvissen voorkomt; 2) de lagen 5 en 6 met diverse typen visuele afferentie; 3) de grenslaag tussen laag 3 en 4, met afferentie uit het telencephalon (de voorhersenen) en uit de torus longitudinalis; en 4) de lagen 3 en 2, met zgn. "diepe" tectale

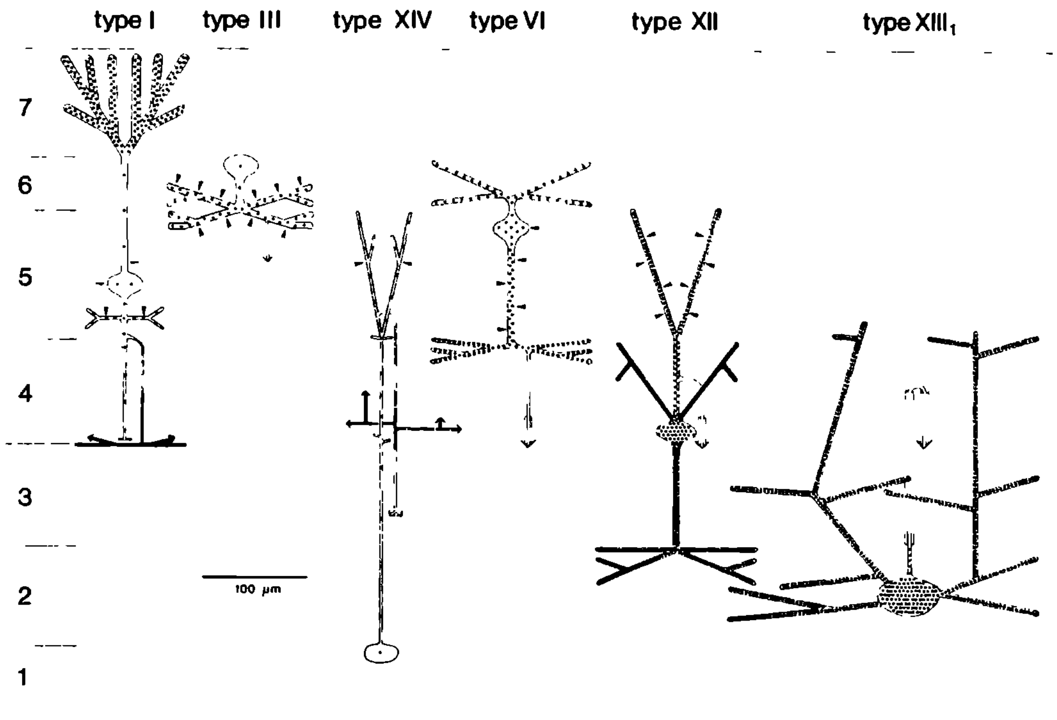


Fig. E. (=Fig. 6 op pag. 103 van het proefschrift). Schematische weergave van het aantal en de verdeling van de synaptische kontakten van zes tectale celtypen. Elke stip vertegenwoordigt 10 synapsen en elke pijlpunt vertegenwoordigt 10 synaptische kontakten met oogzenuwvezels. Witte strukturen met zwarte stippen zijn postsynaptisch zwarte strukturen met witte stippen presynaptisch.

afferentie vanuit diverse hersencentra. In laag 4 eindigen geen afferenten.

Vervolgens is een schatting gemaakt van de mate waarin de tectale celtypen input vanuit deze vier afferente zones verwerken. Deze schatting berust op het laminatiepatroon van presynaptische strukturen enerzijds en postsynaptische strukturen anderzijds, alsmede op de berekende aantallen synapsen voor de onderzochte celtypen. Daarbij is aangenomen dat alle typen presynaptische strukturen in een bepaalde laag synapsen vormen met alle typen postsynaptische strukturen in dezelfde laag. Elk celtype blijkt dan een karakteristieke kombinatie van verschillende typen tectale input te ontvangen, waarbij voor type I torale input het belangrijkst is, voor de typen III, IV, VI, VII en de meeste type XIV cellen visuele input, en voor type XV de "diepe" tectale input. Voor type VIII, IX en X is visuele, torale en telencephale input van min of meer gelijk belang, terwijl de belangrijkste efferente celtypen XII en XIII tectale input uit alle lagen integreren. De laminaire organisatie van het tectum lijkt dus vooral belangrijk te zijn voor multimodale integratie. De tot nu toe gepubliceerde resultaten van elektrofysiologisch onderzoek bieden helaas nog geen houvast voor het toetsen van deze funktionele interpretatie van de struktuur van het tectum, omdat de geregistreerde visuele responsies van tectale neuronen slechts zelden aan een bepaald celtype kunnen worden toegeschreven en omdat er (nog) geen systematisch onderzoek is gedaan naar tectale responsies op andere dan visuele prikkeling.

Uit een overzicht van de literatuur betreffende de tectale efferentie blijkt tenslotte dat het tectum op tenminste tien andere hersencentra projekteert, waarvan de reticulaire formatie in de achterhersenen het belangrijkste gebied is. Zowel type XII als type XIII projekteren naar dit gebied, dat betrokken is bij de regulatie van de motoriek. Het projektiegebied van type VI en type X is nog onduidelijk. Daarnaast blijkt dat sommige type XIV neuronen tot de tectale efferenten gerekend moeten worden, aangezien ze behalve collateralen in het tectum zelf ook collateralen buiten het tectum bezitten.

Konkluderend kan worden gesteld dat de topografische organisatie van het tectum gericht is op het lokaliseren van objekten in de omgeving van het dier en het opwekken van doelgerichte bewegingen ten opzichte hiervan, terwijl de laminaire organisatie van belang is voor het selekteren van relevante objekten op grond van multimodale informatie.

STELLINGEN

I

Een laminaire organisatie van hersencentra impliceert funktionele differentiatie met behoud van topografische relaties

Ebbesson 1980 Cell Tissue Res. 213: 200
Dit proefschrift

II

In gelaagde hersenstrukturen heeft een morfologische klassifikatie van neuronen slechts dan funktionele relevantie wanneer deze in belangrijke mate berust op de positie van dendrieten en axonen in de onderscheiden lagen

III

Voor inzicht in de funktionele anatomie van hersencentra zijn kwantitatieve morfologische gegevens onmisbaar

IV

Toekomstig elektrofysiologisch onderzoek aan het tectum van beenvissen kan zich in eerste instantie het beste richten op het bestuderen van responsies op multimodale stimulatie

Dit proefschrift

V

De hypothese dat de topografische ordening van de retino-tectale projektie berust op chemospecificiteit, is aanvechtbaar

Horder and Martin 1978 Symp. Soc. Exp. Biol. 32: 275
Rager 1980 Adv. Anat. Embryol. Cell Biol. 63
Sharma 1981 Nature 291: 66

VI

De resultaten van Vrensen en Nunes-Cardozo ('81) suggereren dat de werkzaamheid van een synaptisch kontakt niet zo zeer door het oppervlak, als wel door de omtrek van het kontakt wordt bepaald

Vrensen and Nunes-Cardozo 1981 Brain Res. 218: 79

VII

De beperkende faktor bij de regulatie van het aantal kontakten in het centrale zenuwstelsel wordt gevormd door het op dendrieten en somata aanwezige aantal kontaktplaatsen

Cragg 1974 Brit. Med. Bull. 30: 141

VIII

De huidige gang van zaken rond chemisch afval doet het ergste vermoeden over de toekomst van kernafval

IX

In een waterrijk land als Nederland dient het wonen op het water evenzeer geaccepteerd te worden als het wonen op het land

X

Een economie wordt alleen gezond indien produkten, die bij doelmatig gebruik reeds binnen vijf weken bezwijken, niet gelijmd, doch vervangen worden

J. MEEK

Nijmegen, 11 december 1981